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B. MEDICAL SCIENCES

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A CONSIDERATION OF SOME OF THE MODERN THEORIES IN RELATION TO IMMUNITY.

BY PAUL C. FREER.[1]

In opening the past annual meetings of the Association it has been customary for the president to call attention to the advances which have been made in matters relating to the medical sciences in the Philippine Islands during the year just completed, and while my purpose is to discuss considerations relating more closely to scientific studies which may serve to increase the efficiency of prophylaxis in the future, it is not out of place briefly to review what has been accomplished since the last reunion of the members of this association.

The outbreak of cholera which was accompanied by the fear that it would once more take on the dimensions of the ones that have swept the Islands in the past, has disappeared; cases of plague are no longer encountered; smallpox is reduced to a minimum and by reason of efficient vaccination any future serious occurrences of this disease need not be feared; improved individual hygiene among a certain proportion of the population has lessened the frequency of dysentery. Hygienically, therefore, we are in a better condition than we were a year ago. The discovery of the prevalence of trematode infections is another advance which eventually will lead to the adoption of measures destined to bring about at least a partial curtailment of the number of persons affected; studies in the habits and breeding places of the mosquitoes, which have been

[1] The address of the president: Read at the Fourth Annual Meeting of the Philippine Islands Medical Association, Manila, February 27, 1907.

rapidly advanced, will serve as a foundation for plans to combat malaria; and the work done in the study of immunity can not fail to bring with it far-reaching results in the preparation of serums and vaccines. The activity of the veterinary physicians has been such that violent outbreaks of rinderpest, which in certain sections of the Islands threatened a complete destruction of the horned animals, have been prevented by the use of anti-rinderpestic serum, and the methods of preparation of the latter have been markedly improved, so as to increase its efficiency. Work in medical science has also been advanced by the formation of the Army Board for the Study of Tropical Diseases, and by the union of its interests with those of the Bureau of Science. The results of this hearty coöperation are already evident in a series of interesting publications. Work in medical zoölogy has also been undertaken and we expect most fundamental advances in this important branch of tropical medicine during the year to come. The plans for a medical school founded upon modern laboratory instruction and clinics are about completed and we hope, at no distant date, to have an institution which will be as capable as any which may be found in America or Europe of thoroughly training medical students along the lines universally approved to-day.

No doubt then, but that the past year has shown a great advance, and no doubt also but that the next one will be equally prolific of results. We can not stand still, for that in all countries and ages has meant retrogression.

In tropical countries medical studies most peculiarly concern themselves with the diseases of infectious origin. The white man coming here runs the liability of contracting certain of them, the native again is exposed to others, and the best means of limiting and combating the outbreaks which occur have been topics for study in all parts of the world. So seriously do America and Europe take the question of limiting infectious diseases that specially endowed institutions have been established for work in methods of prevention and cure. Great masters in this direction of work have arisen, and far-reaching theories as to the causes of natural immunity to certain diseases in individual races and species and as to the production of artificial immunity have been established as the result of difficult experimental work. The advancement of these theories, their discussion, and the laboratory investigations necessary to prove or disprove the various views has served to advance the cause of the study of immunity in the past few years with a rapidity perhaps equaled by no other branch of science, unless it be that of the physical study of the phenomena of radioactivity. It has been the great service of Ehrlich, of Frankfurt, to explain the existing phenomena by a fundamental view of the causes of immunity which is founded upon chemical considerations and which, to-day, is accepted by the majority of workers in this field and, although it has opponents, even the latter

have been obliged to bring into their considerations conceptions which, while using different terms, still in many respects conform to the position assumed by Ehrlich.

It is with much diffidence that I approach this subject as a chemist. I had hoped when I selected this topic to have some experimental material to bring before the association, but unfortunately my time has been so occupied in other directions that I have been unable to accomplish what I wished. It will therefore be necessary for you to take such discussion as I have to give, for what it is worth.

A merely casual consideration of the phenomena of immunity to disease develops the fact that we encounter specialized resistance to certain types not only in races, but in families, orders, genera, species and even in individuals. By some processes in the development of various groups of animals, some have become resistant to the change in their condition brought about by the introduction of certain foreign cells, others have become immune to other infections, and so on. This resistance may manifest itself either in a destruction of the introduced cells, in an impairment of their functions, or it may appear as a simple tolerance to their presence without deleterious results. Of course, in this connection we must also consider the cell which is introduced, this too can by ages of development have become resistant or tolerant to the influences of certain of its hosts, and in this way may be capable of resisting destruction in their bodies. The main question to be considered is, How is this destruction or tolerance brought about? In the case of the destruction of an introduced cell, this must either take place by the action of some chemical groups or individuals present in the host, by the aid of which the introduced cell is disintegrated and dissolved, or it must be by physical means, by the action of some form of energy by which chemical changes leading to the disintegration of the cell are inaugurated in the latter. That a proper modification of energy can bring about chemical reaction is too well known for discussion, but it is difficult to conceive how an organism which has been produced while subject to all various manifestations of energy which surround us, should, upon being merely transferred to another organism which has also been produced under the same general conditions, be destroyed by such means only. It is true that in the phenomenon of agglutination we may have, at first sight, some indications of the action of polarity, but here also, to produce the change, certain specific chemical bodies must be present. A *change in energy* undoubtedly takes place during the processes of cell destruction or agglutination and this change in energy must have further, far-reaching results, but as yet the study of immunity has not advanced to a sufficient degree to render a consideration of this phase possible. There remains then as the most probable, *a priori* hypothesis the one which assumes that the examples of cell destruction or agglutination which

we observe are brought about by chemical substances present in the host, or by what is still more likely, a combination between certain definite substances in the introduced cell and of others in the host. If now we consider the development of various races from their simplest beginning, we are forced to the conclusion that, in the struggle for existence and in the adaption of individuals to environment, a great variety of substances of gradually increasing complexity must be formed which take part not only in assimilating food but in resisting the encroachments of parasitic organisms. In proportion as, through the ages, the conditions surrounding the race have been altered, as the attacks have multiplied, and the means of resisting them have been applied, there has sprung up the vastly complex chemical system of the higher organisms, and, as the materials which these organisms must assimilate are varied and the means which they must take to resist destruction are also of great variety, it follows that *those chemical bodies which in the host are capable of the destruction of the invader must also be great in number.* It is not possible, considering the specific character of the destruction of certain cells, that one chemical individual should bring about the various types of immunity, indeed, if immunity were due to one chemical individual we would expect it to be universal, and universal immunity would imply the destruction within the body itself of its own tissues, namely, autolysis. Furthermore, the cell which is introduced is in itself resistant in some organisms, non-resistant in others, it may itself therefore have developed immune bodies which must differ from the immune body in the host. We must therefore come to the conclusion that a multitude of chemical groups or individuals must exist in any one organism, which are calculated to resist the invasion of foreign cells. Another question, entirely, is that of the chemical complex or possibly the transferrence of energy which renders the attacking chemical body active. This factor in the destruction of the foreign cell might be uniform throughout, for its action is through the chemical complex which is only one of the destroying factors. If it is simply a method of producing a change of energy it is difficult to conceive why it should act at one time and not at another or, if it is a uniform chemical body, why it should in the sera of certain cases be destroyed at a low temperature and in others at a higher one.

From what has gone before we must conclude that the development of race immunity is a process of heredity, that the substances which confer certain types of immunity on the individuals of a given race have been produced by a course of development concomitant with the other manifestations of evolution and that the chemical bodies which confer the immunity in individuals must be vast in number. In reaching this conclusion we must not lose sight of another means by which immunity can be conferred, namely by the absence of groups capable of uniting with introduced cells or toxins—but this phenomenon also must be the result of heredity.

General considerations of the broader phenomena of immunity and some more special analysis of the production of immunity lead us to a chemical view of the question, and we are then brought to the conclusion that there must exist certain types or groups of complex substances which, coming in contact with the introduced cells, cause their destruction. All investigators are agreed that, no matter how these chemical substances have their origin, whether in the conglomerate of cells which go to make up the body, or in certain special ones, such as the leucocytes, once they are free in the circulation they can unite with the introduced cell and can be removed by it. That this is not a phenomenon of adsorption is proved by the fact that there is selective absorption of the immune bodies or of the toxins by a cell, where two or more of the former are present. All investigators also are agreed that, once the immune substance is fixed by the cell, then the additional action of another body or in the case of the toxin, a chemical group therein present, is necessary to cause the final destruction of the foreign organic complex. As to the nature of this last mode of action, opinions differ.

If we come to consider the actual relative mass of a toxin which suffices to cause the death of an individual, or the actual relative mass of a hæmolytic immune body as compared with that of the corpuscles which it destroys, we are forced to the conclusion that the masses of the reactive bodies are extremely small as compared with those of the cells which they destroy. In an immune serum we have water, salts, fibrin, globulin and in short a great proportion of substances which can not be immune bodies, and this fact must be taken into consideration when we regard the actual quantities of these substances present. But, on the other hand, the cell to be destroyed must act as a whole; that is, for example, if the process of destruction is one of hydrolysis, then the hydrolytic action must disintegrate the whole cell. A guinea pig of 300 grams is killed by 0.0025 cubic centimeter or by approximately 10^{-5} times its mass of diphtheria toxin, a horse by 0.3 cubic centimeter of tetanus and a mouse by 1^{10-7} gram; 2.8^{10-6} gram of tarantula can completely dissolve 200,000,000 red blood corpuscles of the rat. It is true that we can not actually determine, in a given case, just what mass of cells is thrown out of function by a given amount of reagent and just how many cells it is necessary profoundly to disturb before serious consequences result to an organism, and in this respect reactions *in vitro* give us little aid, as here we are not acting under normal conditions of life, and we are also ignorant of the relation between the actual weights of reagents employed. These studies are radically different from the ordinary chemical ones, where the stœchiometric relations can accurately be followed.

However, the apparent disproportion between the mass of the reagent which causes the disintegration of the cells and the probable mass of the

cells destroyed should draw our attention to the phenomenon of catalysis. The study of catalysis and of catalytic reactions has been advanced most markedly within the past few years, and we have come to have a much clearer conception of the principles underlying the changes brought about by catalytic agents than we formerly had. One fundamental fact must always clearly be borne in mind, and that is *the catalyzer can not change the end equilibrium of a reaction, it can only alter its rate.* A simple example will suffice. If we have a mixture of hydrogen and oxygen, the two gases apparently do not unite at ordinary temperatures, some infinitesimal union however does take place with the formation of water, but the rate of reaction is too slow to be measured and it must be derived by interpolation after a study of the change at higher temperatures. However, if the mixture of gases were to remain for a sufficient length of time, centuries in this instance, a final end equilibrium, in which nearly all had been changed to water and but infinitesimal traces of hydrogen and oxygen would remain, would finally be realized. The introduction of a catalyzer, in this case let us say platinum sponge, will so alter the rate of reaction that it may even proceed with explosive violence, a few seconds sufficing to accomplish that which would take ages under ordinary circumstances. The catalyzer itself, however, is not altered. The recent work of Bredig on the colloidal metals has furnished us with a series of inorganic catalyzers which have the remarkable property of being "poisoned" by chemical reagents similar to those which attack the organic enzymes, and even without such a hint as to a possible resemblance between the inorganic colloids and the organic enzymes, it is quite generally believed that the enzymes are specific organic catalyzers with the power of accelerating the rate of normal reactions which otherwise might be infinitely slow.

The theory of Ehrlich, very briefly stated, is as follows: A cell, having certain chemical groups, is introduced into a body. If, in any of the multitudinous cells of that body there is one which has chemical groups capable of uniting with those of the introduced cell, then, on contact, such union takes place. The attacked cell in the living organism, having its equilibrium disturbed by the occupation of one of its chemical groups, proliferates others which, as I will show further on, may or may not be of the same nature and which are thrown off into the circulation. The proliferated chemical substance must be capable of attaching itself to an albuminous molecule of the introduced cell, for it contains the chemical grouping which assumed the original function of binding the introduced cell. But, in itself, this detached group is not able to bring about the destruction of the foreign invader, it must be rendered capable of so doing by uniting with a chemical substance present in the blood plasma, which is termed the complement. The *immune body* which is proliferated therefore is an *amboceptor*, with one group it is capable of

union with the cell, with the other with the complement, and the latter causes the reaction rapidly to take place.

The immune body (or in the case of toxins the haptophore group) has therefore been termed the "sensitizer" which renders the cell capable of destruction by the enzyme-like complement (or toxophore group), and it has been compared to a mordant. This comparison does not seem to me to be aptly chosen, for the fiber and mordant behave toward each other as two colloids of opposite polarity,[2] the mordant is absorbed by the fiber without apparent chemical union, the fiber itself is not thereby rendered more reactive. The mordant acts independently toward the dye and here also we probably have colloidal absorption. The relations between fiber, mordant and dye are therefore largely governed by physical causes. However, in the case of immune body, complement (or toxophore group) and cell the latter is profoundly altered or destroyed—the destruction appearing closely to resemble the processes of hydrolysis, the immune body apparently serving to bring the enzyme-like complement (or toxophore group) in closer space relation to the cell, so that one should scarcely speak of either as the sensitizer, but should rather term the complement the *accelerator*. The change somewhat reminds one of the different stereo-chemical relations present in the formation of lactones from the halogen-substituted fatty acids and some years ago[3] I demonstrated that in these reactions we can have the chemical reactivity influenced by two factors, one taking place through the carbon chain, the other through space. In connection with this view of catalytic action it is interesting to note that Kyes and Sachs[4] in studying the acceleration of the hæmolytic action of cobra venom by lecithin have shown that the more snake venom is used, the less lecithin is necessary to effect complete hæmolysis and *vice versa*, the more lecithin, the less venom need be used for the minimal completely solvent dose.

It will be seen from the above that the proliferation of immune bodies according to this view is brought about by a disturbance of equilibrium within the cell, that therefore the production of immune bodies is a *normal process* accelerated by the introduction of the foreign cell. Under undisturbed conditions of equilibrium these *or similar* immune bodies are also most certainly being given off, but owing to their gradual proliferation, they undoubtedly disappear without causing a change in the normal equilibrium. By the introduction of the foreign cell, however, the *rate of the reaction* is altered so as to throw a large amount of these immune bodies into the circulation at one time. This production of immune bodies, therefore, has much the appearance of catalysis, where the catalyzer which proliferates the immune body is generated within the body cell itself.

[2] W. Biltz: *Ber. d. chem. Ges.* (1904) 37, 1766.
[3] *Ann. Chem.* (Liebig) (1901) 319, 345.
[4] *Berl. Klin. Wchnsch.* (1903), 40, 21.

I have mentioned above that, under undisturbed conditions of equilibrium the immune bodies or *similar substances* are undoubtedly constantly being given off. I had in mind some of the phenomena of organic chemistry in which the change of one stereochemical form into another is brought about, or perhaps more aptly, the alterations which we observe from the keto- to the enol-form and *vice versa.* We have bodies, for example, which when prepared under certain circumstances, react like ketones or aldehydes, these same bodies may spontaneously or under slight stimulus, become altered to unsaturated alcohols and again under others they may at times appear as ketones and at others as alcohols. The same condition may apply when the equilibrium of the living body cell is disturbed by the introduction of the foreign one—the immune bodies which are normally proliferated may be changed in this manner in their chemical structure by the introduced cell so that now they become capable of independent existence and possibly also of firmer union with other cells and so that they are also able to fix the complement. This view does not seem improbable when we consider that we have immune bodies of apparently similar origin, some of which are capable of firm fixation to the introduced cell, others of which are but loosely bound and still others which have their avidity increased by union with the complement.

While I have shown above that the immune bodies may be produced by catalysis of normal processes, it does not follow that they are themselves catalyzers when they unite with the introduced cell, with the toxin, or in the case of toxins themselves, with the body cell, for it has conclusively been shown that such union does take place and that then these bodies are either removed from the serum or that they unite with the toxin. But, if the view of catalysis is correct, them these bodies, when so attached should, at a very slow rate of reaction, accomplish that which they do rapidly under the influence of the complement, for the catalyzer simply *accelerates* an otherwise normal reaction; in other words, to select an example from the phenomena of hæmolysis, the lysis of a red blood cell to which an immune body is attached, should take place very slowly as a normal reaction, which would proceed very rapidly to an end equilibrium by the addition of the complement. Individual test-tube experiments in hæmolysis have not extended over a sufficient period of time to prove or disprove this view, and it would be interesting to follow out this subject if the experimental difficulties are not too great.

On the other hand the complement which is attached to the amboceptor, either permanently, as in the case of the toxins, or which is taken up from the blood serum, as with the hæmolysins, seems much more closely to resemble the catalyzers, but if it is a catalytic agent it must accelerate a reaction which otherwise would take place with extreme slowness, with such slowness, indeed, that without such a catalyzer no noticeable difference of the normal equilibrium would take place. Dr. Strong, acting on this view, has conducted a series of experiments with

diphtheria toxin in guinea pigs which were simultaneously injected with minimal doses of pure hydrocyanic acid, acting on the theory that this substance, which so markedly "poisoned" Bredig's colloidal inorganic catalyzers, would do the same with the organic ones, and that hence the action of the diphtheria toxin should be delayed, with a gradual recovery of virulence as the hydrocyanic acid disappeared. The results were entirely negative. However, this one series proves nothing excepting that other means of experimentation must be resorted to. The work could probably be more successfully carried out *in vitro.*

The process of cell destruction by aid of the complements which are present in the serum has one interesting phase which causes it to differ from that where the catalytic agent, if I may use the term, is a chemical individual which can be isolated, such as lecithin. In the case of the serum complement there is a time of incubation—that is, the reaction proceeds with an increment of increase in unit time—which can only be brought about by the relatively increasing amount of catalyzer present, in other words, the complement must either be *formed during the reaction* or it must be liberated as soon as its work is done, but in the latter event, *in vitro*, we must also have a steadily diminishing amount of substance in unit volume to be acted upon, and a consequent gradual retardation. The study of the *rate* of these reactions and the resulting curves is therefore of fundamental importance, and in the case of hæmolysis the experimental difficulties would not seem to be too great to overcome. It would certainly be well to determine the proportion of complement and immune body as compared with that originally present remaining in hæmolyzed blood after the reaction is complete, although in this connection care must be exercised not to confuse the possible effects of substances like lecithin, which could be separated from the stroma, with the true serum complements.

In closing I wish to call attention to one phase of the researches on immunity which would repay further investigation. Ehrlich has said:

> I accept the existence of haptophore groups exclusively in compounds such as the food stuffs which can enter into the composition of the protoplasm or which, such as the great series of poisonous or not poisonous products of the metabolic changes of living cells, can enter into a combination similar to that of the food stuffs.
>
> The deep-seated difference between the alkaloids, glucosides or medicinal agents of known chemical constitution and those substances which possess haptophore groups and which are therefore capable of liberating antibodies during the process of immunization is shown by the fact that none of the former class have ever been able to give rise to any antibody production of consequence.

However, I believe that this seemingly impassible gulf can be crossed. Undoubtedly, we can place on one side poisons from which no trace of an immunity reaction can be secured and on the other substances, the products of life action, which give all the typical phenomena which

we are accustomed to associate with what we term immunity, but surely the line can not be an abrupt one and hence, in this investigation we must seek chemical individuals of known constitution which, under proper conditions, are capable as synthetic bodies of entering into metabolic reactions. The recent work of Emil Fischer on the amino-acids and the synthetic formation of the polypeptids is suggestive and it is with similar synthetic substances that we may hope to see our next great advance in the study of immunity.

The problems to be encountered in immunization are difficult, they involve painstaking experimental work and close reasoning and thought but, as I have endeavored to show, the modern views of chemistry and physics are all on the side of the worker in immunity; he has but to reason closely to untangle one more skein of the web, and his reward is great. In place of the constant fear of recurring serious epidemics of devastating diseases with their accompanying vast expenses, and in place of the constant vigilance necessary to prevent serious outbreaks of infectious diseases, the worker in immunity may possibly as a result of his studies in the future be able to render a community practically safe from all but negligible sporadic cases. We could then dispense with the rigors of quarantine and its interruption of commerce, or with the enormous loss of life consequent upon the occurrence of epidemics. True, the prejudice against methods of immunization is as yet great, much more of a scientific nature needs to be done, much of a missionary character undertaken, but then not many years ago the very fact of the causation of disease by microörganisms was the subject of the bitterest dispute and many members of the medical profession itself were sceptical as to the results which were to follow. However, the opposition of the profession has practically disappeared, that of the laity will of necessity follow, and our successors will find the way for future advance cleared for them by the pioneers of to-day.

ON THE CULTIVATION OF A BOVINE PIROPLASMA: A PRELIMINARY COMMUNICATION.

By M. Miyajima.

(From the Imperial Institute for the Research of Infectious Diseases, Tokyo, Japan: Director, Prof. S. Kitasato.)[1]

One of the most interesting subjects in medical science, especially in tropical medicine, is an investigation of the parasitic protozoa. So many valuable discoveries have been made in the study of these organisms that it is not necessary here to mention them, but nevertheless at present the knowledge of protozoölogy is not so far advanced as that of bacteriology. This condition may be ascribed to the fact that on the one hand, the life history of the class protozoa is most complicated and on the other, the protozoan parasites can not be cultivated so easily as those of bacterial origin. However, the efforts of several workers have made the study of the pathogenic protozoa more accessible. The mode of transmission of *Piroplasma bigeminum* was clearly demonstrated by Smith and Kilborne;[2] later the relation of mosquitoes to the malarial parasites was discovered. The pure culture of the pathogenic protozoa outside of the living body was first accomplished by Novy and McNeal[3] in the case of *Trypanosoma lewisi.* These fundamental studies have thrown much light upon the methods of investigation in modern protozoölogy. It is my belief that we owe so much to your countrymen in the progress of this science that I have mentioned their admirable investigations in this field before entering upon the discussion of my own subject.

Among the problems in modern protozoölogy which have greatly attracted the attention of scientific men, that one connected with the view of the late Professor Schaudinn[4] with respect to the relation of the hämocytozoa to trypanosomata may be mentioned. Schaudinn announced that the hämocytozoan known as *Halteridium* develops into a trypanosoma in the body of the mosquito; furthermore, he believes that

[1] Read at the Fourth Annual Meeting of the Philippine Islands Medical Association, March 1, 1907.

[2] *Bull. Bureau of An. Industry,* U. S. Dept. of Agr. (1899).

[3] Novy and McNeal: Contrib. to Med. Research (1903), 549.

[4] *Arb. a. d. k. Gsndhtsamte* (1903–04) **20,** 387.

piroplasma may similarly assume a trypanosoma form in the course of its development. However, Novy and McNeal[5] reached a different conclusion from the above observer, for they affirmed definitely that the trypanosomata of birds are not related entirely to hämocytozoa such as *Halteridium* and *Leucocytozoa*.

Again, Rogers[6] succeeded in securing a culture of the small, intracellular parasites of *Kala-azar* known as the Leishman-Donovan bodies, in which the organisms developed into large, motile flagellates, most closely resembling young trypanosomata which had not yet developed an undulating membrane.

Thus, our problem on the relation of hämocytozoa to trypanosomata becomes more complicated and must be settled by further observations. For this reason I took as my material for this investigation a form of bovine piroplasma which is readily accessible in Japan, and made some observations in coöperation with my assistant Dr. Irikura.

Observation 1.—In a paper[7] "On the Piroplasma Found in Japanese Cattle," I have, with Dr. Shibayama, shown that a large percentage of Japanese cattle is infected with a species of piroplasma which seems to be similar to the parasite of coast fever (*Piroplasma parvum*). In the course of an extended study we were able fairly often to demonstrate the same parasites in the blood of the native cattle of Korea. These parasites, found in apparently healthy animals, have been mostly of the small bacillary type, the large pyriform or ring-shaped bodies only being seen occasionally. R. Koch[8] in one of his latest publications, described a peculiar cross-form which distinguishes *Piroplasma parvum* from its allied parasites. After a prolonged search this form was also encountered in the blood smears prepared from our native cattle, although only in small numbers (Pl. I, fig. 1). However, we have not as yet observed any symptoms characteristic of coast fever in our infected Japanese cattle, although the parasites found therein might morphologically be identical with *Piroplasma parvum* Theiler. There are diverse views in regard to the pyriform body of this species. Some observers believe that the pyriform body occasionaly met with in the blood of an animal suffering from coast fever rather represents a mixed infection with "Texas fever," while others consider it to be a distinct form occurring in the life cycle of *Piroplasma parvum*, as is the case in the other varieties of this group. In support of the latter view it may be stated that we have observed the large pyriform body occurring regularly, although in small numbers, in the circulating blood of every infected animal, and in Japan an infection with Texas fever is out of the question.

[5] *Am. Med.* (1904), **8**, 932.
[6] *Quart. J. Micr. Sc.* Lond. (1905), **48**, 367.
[7] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1906) **54**, 189.
[8] *Deutsche med. Wchnsch.* (1905), **47**, 1865.

As is already known, the parasite *Piroplasma parvum* is also characterized by the fact that the infection can not be transferred by the inoculation of the blood itself, and in this respect we have observed that it differs greatly from its allied organisms, *P. bigeminum, P. carus,* etc.; however, on the other it shows a great similarity to "Hæmoprotens" which is a common parasite in the blood of birds.

Observation 2.—Since the discovery of the important rôle taken by ticks in infections with piroplasmata, many observers have in vain endeavored to prove the existence of the developmental forms in the bodies of the ticks which live on infected animals. Very recently an actual demonstration of a developmental change of the piroplasma was given for the first time by R. Koch.[9] He described certain developmental forms of *Piroplasma bigeminum* and *P. parvum,* the final stages of which are as yet unknown. After his work was published, we endeavored to repeat his experiments by feeding the cattle tick *Rhipicephalus australis,* which is found abundantly on the native cattle of Japan and is regarded as being a probable carrier of *Piroplasma parvum.* After several unsuccessful trials, my efforts were directed toward the cultural method by which Rogers and other observers were able successfully to demonstrate the flagellate stage of the Leishman-Donovan bodies.

The following different culture media were tested in our preliminary experiments on the cultivation of the piroplasma; blood agar, sodium citrate, both acidulated and nonacidulated; beef extract, peptone water; calf's serum; physiologic salt solution; common bouillon, etc. On the 4th of July, 1906, we first observed a few motile organisms in one test tube which contained a small amount of infected blood mixed with acidulated sodium citrate solution, prepared according to the method of Rogers. The entire series of cultures was then carefully examined but no evidence of development of any motile organism was obtained except in the bouillon ones. This medium was apparently the most favorable, large flagellates having abundantly developed therein after four days' incubation at a room temperature. Only a slight multiplication of the organisms took place in the first culture which we obtained in acidulated sodium citrate.

The length of the organism at its full-grown stage, as it is observed in cultures, is about five times the diameter of an erythrocyte. It, at this time, possesses a well-defined undulating membrane and a long flagellum. The position of a nucleus and blepharoplast in the body of the flagellate renders it impossible to distinguish it from a typical trypanosoma developed in a culture. (Pl. III, fig. 2.)

The method which we have employed is a simple one, and is practically

[9] *Ibid* (1905) 47, 1865, and *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1906) 54, 1.

the same as that used by Rogers.[10] The blood containing intracellular parasites, is drawn from the jugular vein and then quickly defibrinated under strict precautions so as to avoid bacterial contamination; it is then directly mixed with ordinary nutrient bouillon in proportions varying from one-fifth to one-tenth, and placed aseptically in sterile test-tubes which thereafter are maintained at a temperature of 20° to 30° C. The development of the parasites in a successful culture takes place in the following manner: On the first day no motile form is seen; on the second, there can be observed a certain number of peculiar cells which occupy the upper layer of sedimented corpuscles and which macroscopically appear as a series of whitish dots. Very few motile forms resembling typical trypanosomata are visible in these cells on the third day after incubation, but thereafter the trypanosomata multiply vigorously and reach the maximum number between the tenth and fourteenth day. (Pl. III, fig. 12.)

In a culture kept at room temperature, the trypanosomata remain motile until forty-five days later, at this time most of them have undergone degeneration and globular cells with irregular granulations result. In a culture preserved at a lower temperature, ranging from 10° to 20° C., the organism on the contrary remains alive until three months after the maximum number has been reached. It is noteworthy that subcultures are also readily obtained by inoculating from the original strain into a new blood bouillon, as in the case of *Trypanosoma lewisi*.

The most important factor in securing the multiplication of the parasites essentially consists in great precautions in avoiding the slightest contamination with bacteria, as is the case with other cultures of protozoa.

We have already made miscroscopical examinations of the blood in over 200 cattle and among them not one has proved to be infected with trypanosomata; moreover, all varieties of bovine trypanosomiasis are entirely unknown in our country, at least up to the present time no one has demonstrated a case of such infection.

As is well known, there are many protozoan parasites which infect a living host without the latter manifesting any pathological symptoms; it is also a fact that microscopic examination is not as delicate a means for the detection of a small number of parasites as is the cultural method, which is essentially used in the study of bacteria. From a consideration of these conditions it seems more natural at once to consider the trypanosoma-like flagellates found in our cultures to be true trypanosomata, which, owing to their extremely small number in the original blood, would fail of detection by means of the microscopical examination alone.

However, the relation between piroplasma and trypanosoma, although supported by the views of Schaudinn and other observers, is still a question

[10] *Lancet* (1905) 1, 1684.

of much interest and one which needs further investigation and therefore our observations on the parasites were extended further. The results of this study are here presented in as brief a manner as possible.

Observation 3.—Twenty-one native cattle were carefully examined both by microscopic and cultural methods at the same time. Of this number, nine were shown by microscopic examination to be infected with *Piroplasma parvum;* in only seven of these cases were the flagellate organisms found in the cultures; in the remaining two, the cultures gave no growth of flagellates. Microscopic tests failed to detect any parasite in the other twelve cases and, similarly, the cultural method gave a negative result. Therefore, the almost constant occurrence of piroplasma in the blood and of flagellates in the culture renders the existence of certain relations between them very probable and we can hardly regard this occurrence as a merely accidental phenomenon.

I have also ascertained the minimum doses of infected blood which would give a growth of the flagellates in cultures. For this purpose a freshly prepared culture medium was used, which consisted of the blood of an uninfected calf or rabbit and common bouillon. Each tube of the blood-bouillon was inoculated with a different amount of the infected blood which harbored a fair number of intraglobular parasites. I have obtained some interesting results from a series of experiments along this line, namely, that the quantity of the incubated material is a matter of indifference in respect to the development of trypanosomata in the cultures, since one platinum-loop full of the blood gave exactly the same result as if one cubic centimeter of the same material was used. In other words, the germ which gives rise to trypanosomata in the culture exists even in so small an amount of the blood as one platinum loop-full, or one drop. These facts suggested a possible means of detecting the flagellates by direct blood examination, if they exist originally in the blood of an infected calf. However, a large number of blood preparations, both fresh and stained, were thoroughly investigated, but no flagellates were observed. This mode of observation was *repeated* several times, but every series gave negative results. Furthermore, we have centrifugated a mixture of the infected blood and salt-solution at a low temperature, to prevent a possible development of the parasites. The sediment was than removed drop by drop from the upper layer of the centrifugated material and subjected to careful microscopic examination. It was expected by this procedure to obtain direct evidence of the existence of trypanosomata in the blood, but our trials failed to detect any motile or flagellated organism.

Observation 4.—The most effective evidence in favor of the view that the flagellates originate in the culture may be secured by a morphologic study of each developmental stage of the parasites, but many difficulties are encountered in such investigations. On the one hand the scanty

number of the pyriform bodies in the material treated renders the work most difficult and on the other, when a flagellate once starts to develop in the culture, it multiplies so quickly as very much to interfere with the work of tracing each developmental stage. After extensive researches, certain interesting forms of the parasites were found in the stained films prepared from young cultures, which afterwards gave a growth of numerous trypanosomata. Soon after the culture was made, a few round cells, which morphologically were identical with the free pyriform bodies, were seen in stained preparations. (Pl. I, fig. 2.) Within the first twenty-four hours after incubation, the diameter of the round cells (Pl. I, fig. 3) increased, and it finally became twice that of an erythrocyte. Associated with the enlarged cells there also were irregular amœboid forms (Pl. I, fig. 4) similar to the former in nature. In this stage the chromatin of the cells was distributed more or less irregularly in the cytoplasm; in some it was diffused throughout, while in others, distinct chromatin granules were demonstrable. A further advanced stage of the parasites which occurred in cultures was a vacuolated, globular form which retained its staining property just as nonvacuolated cells do. (Pl. II, fig. 5.) As a result of the distension of the vacuole, the parasite gradually assumed the shape of a large ring, of which the thickest part contained some chromatic dust. (Pl. II, fig. 6.) In addition to these, there were many degenerated cells of an allied nature, which were principally characterized by the numerous vacuoles and hypertrophic chromatin. (Pl. II, fig. 7.) The change which took place within the next twenty-four hours was very interesting. The ring-shaped cells (Pl. II, fig. 8) in which the chromatic dust had already become rearranged so as to form a large nucleus and a small blepharoblast, transformed themselves into spindle-shaped organisms in which no visible flagella were present. Associated with this form was observed the flagellated motile parasite (Pl. III, fig. 9) which morphologically was almost identical with the former.

Finally, in the 72-hour culture we obtained a flagellated form showing the development of an undulating membrane. The size of this form, which was that of a typical trypanosoma, increased and afterwards it multiplied in the manner usual for true trypanosomata—that is, by longitudinal fission (Pl. III, figs. 10 and 11)—thereafter, as has already been mentioned, the fullgrown and divisional forms increased in number.[11]

The recent observation of R. Koch[12] in regard to the developmental changes of piroplasma are of great interest, for he described several forms encountered in the body of ticks which in some respects resemble those obtained by us in culture. In the first place, his most predominating

[11] A detailed account of these developmental forms of *Piroplasma parvum* must be left for our more complete paper on the subject.

[12] *Deutsche Med. Wchnsch.* (1905), 47, 1865.

forms are peculiar cells, provided with numerous fine, protoplasmic processes; furthermore, he described a large, rounded form and a club-shaped parasite which closely resembled the oökinetes of *Plasmodium* and *Halteridium*. These important observations were later confirmed by Kleine[13] who used a different method of investigation. He secured a culture of canine piroplasma by diluting the infected blood with salt solution and was also able to demonstrate the peculiar, star-shaped cells and globular forms in his cultures. The further developmental forms, after the majority of the cells had undergone degenerative change, did not appear in cultures of *Piroplasma canis*.

In comparing the results obtained by both of these authors with our own, we find many points of resemblance in respect to the morphological features of the parasites. The star-shaped forms described by Koch and Kleine probably correspond to the amœboid cells (Pl. I, fig. 4) found in the early developmental stage of our parasite, though the latter does not possess such a fine plasmic process as the former. The globular cells (Pl. I, fig. 3; Pl. II, figs. 5 and 6) are a common form occurring in the development of all cases, and the club-shaped ones figured by Koch seem to correspond to the first stage of the motile flagellate observed with our parasite. On the other hand, the successful culture of the intracellular parasite was first accomplished by Rogers with the Leishman-Donovan bodies. The facts demonstrated by the above-mentioned authors therefore greatly support our own observations. It may be well here to add that the mode of formation of a flagellated form in the case of our parasite is apparently different from that of others; especially does it differ from that of the *Leishmania donovani*, which transforms to the flagellate by direct elongation of an enlarged globular form, whereas the piroplasma, according to our observations, develops in the unusual manner described above.

Observation 5.—The final means by which we are able to reach a definite conclusion in respect to the trypanosomata consists of animal experiments with the cultures. It is a well-known fact that with the parasite of coast fever (*Piroplasma parvum*) the direct inoculation of the blood into cattle, even in a large amount, always fails to give rise to infection with the appearance of parasites in the animal so inoculated, and as regards this point the parasites investigated by us were already proved to be identical with *Piroplasma parvum*. We selected three calves which, after repeated examinations by microscopic and cultural methods, were demonstrated to be free from parasites. The animals were then inoculated with a culture containing motile trypanosomata in abundance and as a necessary prerequisite to the successful completion of the experiment, they were kept under such conditions that danger of infection from any source, especially from ticks, was avoided. The blood

[13] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1906), 54, 10.

of the calves, after the injection, was microscopically and culturally examined from time to time. One of the animals proved itself to be refractive, as is observed in the case of the direct inoculation of the infected blood. However, the others were infected with parasites, which fact was first proved by the cultural method and later demonstrated to be the case microscopically. In one instance, eight days after inoculation, the blood of the susceptible animal began to give a growth of the flagellated parasite in culture, whereas seventeen days later by the aid of the microscope alone, the intracellular parasites were visible in the same animal. (Pl. III, fig. 12.) The number of our experiments on animals is not as yet sufficiently large for us definitely to give the duration of incubation of the infection and other details.

The foregoing discussion brings us to the following conclusions:

1. A variety of hämocytozoa known as *Piroplasma parvum* can readily be cultivated outside of the living body.

2. The parasites undergo the developmental change in blood-bouillon and finally take the form of a typical trypanosoma. This trypanosoma can not be detected in the blood of infected animals.

3. A simple mixture of blood and bouillon is the most suitable medium for the cultivation of protozoa such as *Piroplasma parvum* and *Trypanosoma lewisi*.

ILLUSTRATIONS.

[Photomicrographs by Mr. Charles Martin, photographer of Bureau of Science, Manila.]

PLATE I.

FIG. 1. Blood smear from a calf inoculated with a culture containing many trypanosomata; a typical cross-shaped form and many intraglobular parasites. Mag about 1050×1.

2. Swelling of intracellular parasite after the blood is drawn from a naturally infected calf. Mag. 1570×1.

3. Extracellular globular parasite in a three hours' culture at 25° C. Mag. 1570.

4. Amœboid parasite in a three hours' culture at 25° to 30° C. Mag. 1580.

PLATE II.

FIG. 5. Large, globular parasite with two small vacuoles developed in twenty hours' culture at 25° to 30° C. Mag. 1570.

6. Large globular forms with one large vacuole developed in twenty hours' culture at 25° C. Mag. 1570.

7. Irregular, large, vacuolated forms occurring in forty-eight hours' culture at 25° C. Mag. 1100.

8. More advanced stage in the development of the globular parasite. Body crescentic. Forty-eight hours' culture at 25° C. Mag. 1305.

PLATE III.

FIG. 9. Flagellated form occurring in a forty-eight hours' culture at 25° to 30° C. Mag. 1570.

10. Enlarged flagellate possessing two blepharoplasts and flagella with but a single nucleus; preliminary divisional form occurring in three days' culture at 25°. Mag. 1305.

11. Smear from a four days' culture grown at room temperature (20° to 27° C.), showing (*a*) typical, dividing trypanosoma; (*b*) a slender flagellated form. Mag. 1305.

12. Smear from seven days' culture incubated at room temperature (20° to 27° C.) Showing numerous trypanosomata. Mag. 820.

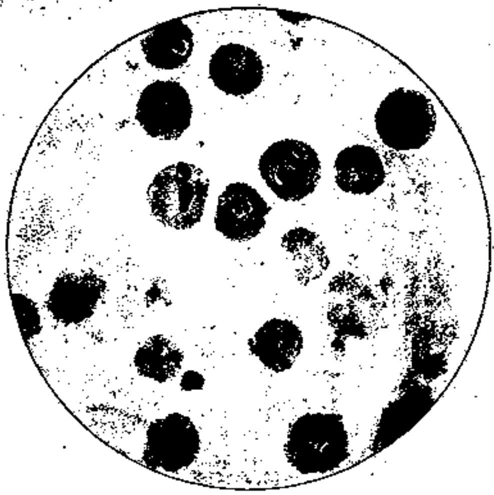

FIG. 1.

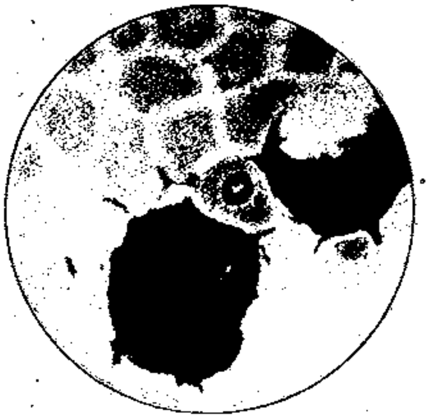

FIG. 2.

FIG. 3.

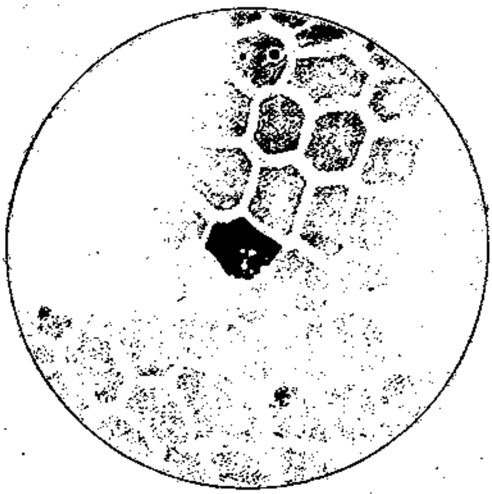

FIG. 4.

PLATE I.

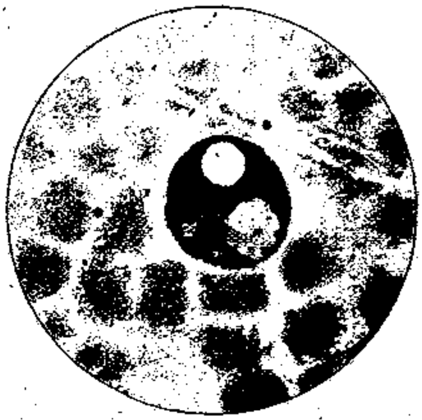

FIG. 5.

FIG. 6.

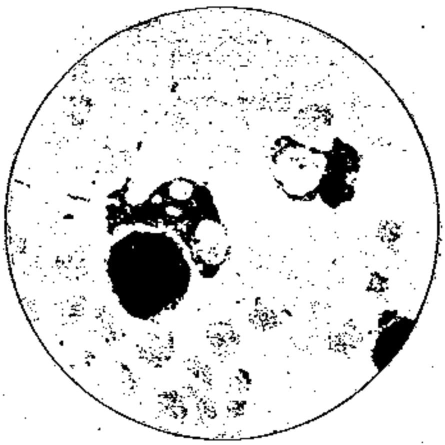

FIG. 7.

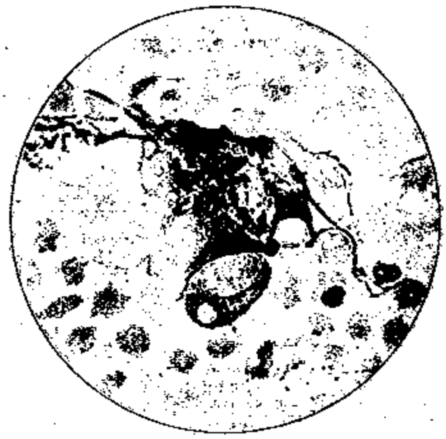

FIG. 8.

PLATE II.

EXPERIMENTAL INVESTIGATIONS REGARDING THE ETIOLOGY OF DENGUE FEVER, WITH A GENERAL CONSIDERATION OF THE DISEASE.[1]

By P. M. Ashburn and Charles F. Craig.[2]

(From the Laboratory of the United States Army Board for the Study of Tropical Diseases, Division Hospital, Manila, P. I., and the Biological Laboratory of the Bureau of Science.)

CONTENTS.

[1] Read by abstract at the Fourth Annual Meeting of the Philippine Islands Medical Association, March 1, 1907.

[2] P. M. Ashburn, captain and assistant surgeon, United States Army, and Charles F. Craig, first lieutenant and assistant surgeon, United States Army, constituting the United States Army Board for the Study of Tropical Diseases as they Occur in the Philippine Islands.

I. INTRODUCTION.

Having been instructed by the Surgeon-General of the Army to investigate the cause of dengue and to determine the possibility of the transmission of the disease by mosquitoes, we undertook the experiments to be detailed below when the opportunity was afforded us by the occurrence of the disease in epidemic form at Fort William McKinley, Province of Rizal, Luzon, P. I., 5 miles out of Manila.

The incidental investigation of available literature on the subject indicated to our minds that a brief discussion of all the features and phases of the disease would not be out of place, as there are discrepancies to be reconciled and certain doubts to be settled, some of which may possibly be ended by our conclusions, while on the other hand some points on which our experiments leave us in doubt seem to be strengthened by the history of the disease as recorded in the literature.

The literature has not been nearly as accessible to us as we could wish, and several articles, both old and new, which we desired to consult, were not available.

The first question that merits discussion in the consideration of this disease concerns its existence as a disease entity. At times the question of identity with influenza has been raised.

However, in our opinion, the study of the writings of those men who have had experience with dengue should promptly and finally settle the question. The agreement in the descriptions from 1827 and 1828, when Dickson, Squaer, Osgood, Dumaresq and others wrote of the disease, down to the latest articles accessible, such as those of Guiteras and Cartaya, is remarkable, while the almost simultaneous appearance from opposite sides of the world of two reports agreeing so closely throughout as do those of the Australian committee and of Guiteras and Cartaya, is even more striking, and the clinical picture drawn from these writings from almost a century of literature and from every part of the tropical and subtropical world is quite distinct and unique. It has very little in common with the picture of influenza, and many points of difference, and in our opinion it would be only the exceptional case that would give rise to confusion.

II. EPIDEMIOLOGY.

1. HISTORY.

The written history of dengue extends back clearly as far as 1827, while it is quite probable that it was recognized and described much earlier: By Rush (1), in 1780, as "bilious remittent fever;" by Boylon, in Java, in 1779, while Thomas (3), speaks of Pazzio describing an epidemic in Seville, in 1764–68, which was traced to Africa. Dickson's (2) writings, however, first brought the question into prominence, at least with Americans, and his observations and descriptions are applicable to the disease as it occurs to-day.

The number of writers, from the earliest down to the present, who have likened the disease, in its causation and spread, to yellow fever, is very striking.

Foster says that most of them have considered it a modified yellow fever. We do not so infer from such literature as we have seen. They speak of it as having a common cause, but as these statements were made before the specific causes of any of the fevers were known, we take it that the writers meant merely that the cause of both was a "miasm," an atmospheric condition, the state of the ground water, etc. We have not found any author who states that the one disease may arise from the other.

The disease has occurred in most widespread epidemics in the tropical and subtropical world, not only covering large areas of country, but attacking a greater proportion of the inhabitants than probably any other known disease, and usually also affecting most of the other members of a family after one has been attacked. That being the case, it is rather surprising that a belief in its contagiousness has not been practically universal.

As a matter of fact, only a minority of the authorities whose writings are accessible to us have expressed an unqualified belief in its contagiousness, while many have rejected it entirely.

Dickson considered it very highly contagious, while many of his contemporaries liken it to yellow fever in its manner of progression. Most writers express themselves as in doubt on the subject. Wragg denied contagiousness, as did Horlbeck (4).

Holliday (6) wrote to sixty practitioners who had large experience with the disease, asking their opinions as to its contagiousness. Forty-five replied that it was simply epidemic, four that it was contagious, two were in doubt, and the rest did not answer.

One point on which the writers, from first to last, almost unanimously agree is the influence of atmospheric conditions. Hot, sultry weather, with abundant rains, is by all thought to favor the occurrence of epidemics. Nearly all also agree in stating that lowlands, seaports, the deltas of rivers and the neighborhood of marshes, are favorable places for the occurrence of the disease, while it seldom prevails extensively inland, and almost never at high altitudes.

If, in addition to this, we consider that it is a tropical disease, only extending as far north as our Gulf States and Charleston in the summer, usually about August, and dying out with the coming of frost (6), we have one of the reasons for likening it to yellow fever. Epidemics have possibly occurred in Philadelphia in 1780, and Ohio, in 1828, though we have not had access to the original accounts of these outbreaks, but it was only in the hottest part of the year in each instance.

Guiteras and Cartaya (7) say: "Dengue presents in its epidemiology a great resemblance to mosquito-borne disease, especially yellow fever. Epidemics appear in the hot season, even in the Tropics. It selects by preference the great seaports, the coast, and avoids the interior highlands. We meet places in which infection persists, epidemics which develop slowly in the beginning. If it then spreads rapidly it is because of the shortness of the incubation and the presence of many susceptible people. * * * Everything indicates that its transmission is not direct from sick to well. * * * It is common for a sick person to be taken to a place where dengue does not exist, and no harm result. * * * In Havana itself, and at the very height of the epidemic, there was a place in which the disease did not spread, simply because of freedom from insects. In Las Animas (Hospital) we have treated a good number of cases in the same rooms with other patients * * * without any contracting dengue. We have treated the greater part of our cases in the pavilion used for infectious diseases of known means of transmission, a pavilion protected against insects by metallic screening and frequent fumigation to kill mosquitoes, but where other disinfection was not practiced, and still dengue has not been transmitted to anyone."

They also speak of some experiments they made on mosquito transmission, but they do not publish the work, and state that they attach little value to it.

Cazamain (8), in reporting an epidemic on the French ship *Kersaint*, suggests the possibility of the mosquito acting as a "carrier."

The Australian committee (Robertson) report (9) says: "It is undoubtedly highly infectious, but clinical observation does not enable us to form any definite opinion as to the mode in which the infection is carried from person to person. In some respects the spread of the disease suggests some peculiarity in the method of propagation differing from that of the well-known diseases, influenza, scarlet fever, measles, etc. It appeared to spread particularly to contiguous houses, whole streets being attacked *seriatim*. One observation appears to have some bearing on the method of propagation. That is, the medical men have, in many instances, appeared immune to the disease, although exposed to it daily, indeed hourly, until cases appeared in their own households, when they fell victims, being usually among the last members of the households to be attacked."

Hirsch (10) says that its infectiousness is certain, its contagiousness doubtful, but he gives some significant quotations that suggest mosquito transmission. Thus he quotes Waring (North Amer. M. & S. Jour., 1830) as saying of the epidemic of 1826, "the breakbone fever has been suppressed by the frost," and of that of 1828, "it terminates under the effect of frost." Arnold (11) he quotes as follows: "This disease is undoubtedly affected by frost. The diminution of cases last fall was as marked as the diminution in our endemic climate [yellow] fever usually is."

Speaking of the Madras epidemic of 1872 Hirsch says that it ended in the middle of October with the onset of strong winds and colder weather. He also cites instances to show that freedom from epidemics is conferred by altitude.

Leichtenstern (12), although regarding the disease as contagious, makes the following suggestive observations concerning it:

1. It is a disease of tropical and subtropical zones, and in these zones it has a marked preference for the hot season, and almost always ends, as if suddenly cut off, on the recurrence of cold weather or the beginning of the cool season.

2. It is a disease of sea coasts and ports, and coast cities, and may go up large navigable streams, as the Mississippi. It rarely occurs inland or at high altitudes. When it went 4,000 feet high on Lebanon in 1889 the season was exceptionally hot.

3. He considers it a "highly contagious" disease, carried by ships, pilgrims and emigrants; nevertheless, contagion alone, in the strict sense of the term, can not satisfactorily explain its occurrence and spread.

4. He calls it contagious-miasmatic; that is, it is contagious from person to person, but only under certain conditions of time and place.

5. He likens its manner of spread (he wrote prior to the work of Reed, Carroll, *et al.*) to that of yellow fever.

Sandwith (13), writing of the disease in Egypt, says it always begins in August and September, with the rains, and ends in December, when colder weather begins. In speaking of its relation to surface water he says: "The conclusion would seem to be that dengue, with its unexplained affection for coasts, rainy seasons and large rivers, only appears in Egypt when the Nile is in its annual flood." He believed the disease to be contagious but cites instances that appear to point to some other method of diffusion.

Smart (14) likened the disease in some ways to yellow fever but said: "There is sufficient evidence of its infectious, if not of its contagious properties."

Fayrer (15) says: "The degree of communicability as well as the work of communication can, however, hardly be regarded as a settled question." He quotes Charles as expressing "his belief that when the eruption is out the danger

of communicability is greatest and that on or about the tenth day, when the eruption has disappeared and after carbolic-acid baths, the danger of communication ceases." We think that this observation is probably correct, but explainable quite apart from any belief in direct contagion.

Fayrer quotes many Indian medical officers who express most diverse views concerning the epidemiology of the disease, yet nearly all seem to be readily accounted for under a theory of mosquito transmission.

Manson (16) does not express himself as believing in any special method of propagation, though he quotes Graham's work.

Van der Burg (17) says that epidemics are most common in the hot, moist season, and cease when the weather grows cooler, especially if very heavy rains occur, accompanied by cool winds. He thinks that dengue is very probably contagious, but that the contagious principle is different in character from that of smallpox, epidemic influenza and like diseases. He states that unknown exogenous conditions are necessary for its spread, wherefore many regard it as a "*contagious-miasmatic disease.*"

Scheube (18) says that the question of contagion is still undecided, but he considers the disease one requiring unusual conditions for its spread.

Agramonte (19) expresses the belief that the disease may exist in a latent state for weeks, under conditions not as yet well recognized.

Stitt (20) says that the disease is not infectious in the same sense as is influenza.

Harris Graham (21) in 1903 expressed his definite belief that the disease is transmitted by mosquitoes, and cited some interesting experiments in support of his belief. This belief seemed well founded, and the experiments in support of it almost conclusive, but the value of his work was impaired by the fact that in the same paper he described as the etiologic factor an intracorpuscular "organism with amœboid movement, and in many ways resembling the *Plasmodium malariæ.*"

It is probable that many workers, having satisfied themselves that such an organism did not exist in the blood of dengue, concluded that Graham had worked with some other disease, and so did not recognize the real importance of his work. We are unable to believe that the disease is characterized by the presence of any organism microscopically resembling that of malaria, but we think that our observations do lend support to Graham's conclusions as to mosquito transmission.

Carpenter and Sutton (22) failed to transmit the disease by means of mosquito bites, but we think that we can explain their failure more readily than we can the negative results which we obtained in some of our own experiments.

2. THE FORT WILLIAM M'KINLEY EPIDEMIC.

The epidemic from which we drew our cases is of great interest, as we think that its history fully supports our belief that the disease is mosquito-borne. Fort William McKinley is situated about 5 miles from the city of Manila, upon rolling land consisting of slight elevations interspersed with low, damp country. There are stationed at this post two regiments of infantry, two squadrons of cavalry, one battery of field artillery and one company of engineers.

The infantry barracks are situated near a small stream which drains the lowest part of the post in their vicinity. This stream is overgrown with a rank tropical vegetation and is an ideal breeding-place for mosquitoes. The epidemic began in the barracks of the Sixteenth Infantry, which is situated nearer this stream than any of the other barracks

of the post. From the Sixteenth Infantry the infection spread to the Thirteenth, and from the Thirteenth to the contiguous barracks of the Eighth Cavalry. It is significant that the battery of Field Artillery and the company of United States Engineers almost entirely escaped the infection, and that their barracks are situated at least 2 miles from this stream, upon high, well-drained land.

The tables, and map which follow show the manner in which the disease spread from barrack to barrack and through the post. The stream mentioned is indicated upon the map in red.

Table illustrating the spread of dengue fever at Fort William McKinley from July 1, 1906, to November 1, 1906.

SIXTEENTH INFANTRY.

Organization and date.	No. of cases.	Organization and date.	No. of cases.	Organization and date.	No. of cases.	Organization and date.	No. of cases.
Company A	20	Company C	25	Company D—Continued.		Company K[a]	
July 1	1	July 2	2	October 28	1	Company L	24
July 2	1	July 12	2	Company E	1	July 13	1
July 3	1	July 16	1	July 22	1	July 15	1
July 8	1	July 18	1	Company F	3	July 17	1
July 9	1	July 20	1	July 17	1	July 18	1
July 10	1	July 21	1	August 5	1	July 22	1
July 12	1	July 22	1	August 9	1	July 24	1
July 13	2	July 23	1	Company G	7	July 25	1
July 15	2	July 24	1	July 20	1	July 27	1
July 17	1	July 25	3	July 23	1	July 30	1
July 18	1	July 26	2	August 6	1	August 1	1
July 22	2	July 28	2	August 7	1	August 5	1
July 25	1	August 1	1	August 8	1	August 6	2
July 27	1	August 2	2	August 9	2	August 9	1
July 30	1	August 5	1	Company H	8	August 11	4
August 1	1	August 11	1	July 18	1	August 14	1
August 7	1	August 16	1	July 24	1	August 20	1
Company B	26	August 24	1	August 2	2	September 9	1
July 7	1	Company D	24	August 4	1	September 10	1
July 9	1	July 5	1	August 5	1	September 14	1
July 10	2	July 12	1	August 7	2	September 20	1
July 11	2	July 14	2	Company I	12	Company M	8
July 12	2	July 16	1	July 26	1	July 25	1
July 13	4	July 17	1	July 30	1	July 30	1
July 15	1	July 18	4	August 4	1	August 3	1
July 16	1	July 19	3	August 9	1	August 7	2
July 17	1	July 20	1	August 14	1	August 24	1
July 18	1	July 23	1	August 15	1	August 28	1
July 19	1	July 26	1	August 18	1	August 29	1
July 20	1	July 27	1	August 19	1	Band	3
July 21	1	July 29	1	August 21	2	July 29	1
July 22	1	August 1	1	August 22	2	September 11	1
July 23	3	August 4	1			October 6	1
July 27	1	August 7	1			Total	161
August 1	1	August 8	1				
August 3	1	September 10	1				

[a] Not on duty at post.

Table illustrating the spread of dengue fever at Fort William McKinley from July 1, 1906, to November 1, 1906—Continued.

THIRTEENTH INFANTRY.

Organization and date.	No. of cases.
Company A	4
July 29	1
July 30	1
August 4	1
August 9	1
Company B	19
July 12	2
July 16	1
July 17	1
July 22	1
July 24	1
July 27	3
July 28	1
July 29	4
July 30	1
August 1	3
August 9	1
Company C	19
July 7	1
July 10	1
July 12	1
July 14	2
July 15	4
July 18	2
July 19	2
July 22	1
July 29	1
July 30	1
August 2	1
August 6	1
August 12	1
Company D	26
July 13	1
July 15	1
July 16	1
July 17	2
July 27	1
July 28	3
July 29	8
July 30	2
August 1	1
August 4	3
August 6	1
August 11	2
Company E	16
August 14	1
September 12	1
September 21	1
September 26	2
September 27	1
Company E—Continued.	
October 2	1
October 6	1
October 10	1
October 12	1
October 24	1
October 27	1
October 28	3
October 31	1
Company F	7
August 4	1
August 22	1
August 30	1
September 6	1
September 16	1
September 26	1
September 29	1
Company G	8
August 7	1
August 9	1
August 10	1
September 2	1
September 5	1
September 10	1
September 29	1
October 11	1
Company H	35
August 11	1
August 18	1
August 19	3
August 27	1
September 2	1
September 3	1
September 4	2
September 6	1
September 7	1
September 9	2
September 11	1
September 18	1
September 19	1
September 20	1
September 21	1
September 22	2
September 26	2
September 28	3
September 29	3
September 30	1
October 1	1
October 8	1
Company H—Continued.	
October 10	1
October 12	1
October 20	1
Company I	23
July 27	1
July 29	1
August 1	1
August 4	2
August 15	1
August 19	1
August 22	1
August 26	1
August 28	1
August 30	1
September 3	2
September 4	1
September 6	1
September 8	1
September 10	1
September 12	1
September 13	1
September 16	1
September 20	1
September 30	1
October 25	1
Company K	35
July 14	1
July 22	1
July 25	2
July 27	1
July 29	1
August 1	1
August 2	1
August 4	2
August 8	1
August 10	1
August 11	1
August 13	1
August 16	2
August 17	1
August 20	2
August 22	1
August 24	1
August 26	1
August 29	2
September 4	5
September 5	1
September 6	1
September 8	1
Company K—Continued.	
September 10	1
September 20	1
October 17	1
Company L	27
July 13	1
July 15	3
August 1	2
August 2	1
August 8	1
August 11	1
August 26	1
September 1	3
September 2	1
September 3	1
September 4	1
September 5	1
September 6	1
September 10	1
September 12	1
September 16	1
September 18	1
September 19	1
September 24	1
September 26	1
October 2	1
October 5	1
Company M	11
July 15	1
July 29	1
August 2	1
August 4	2
August 17	1
August 20	1
August 26	1
September 18	1
September 23	1
October 25	1
Band	8
July 18	1
August 8	1
August 11	1
August 13	1
September 18	1
October 1	1
October 15	1
October 24	1
Total	238

Table illustrating the spread of dengue fever at Fort William McKinley from July 1, 1906, to November 1, 1906—Continued.

EIGHTH CAVALRY.

Organization and date.	No. of cases.	Organization and date.	No. of cases.	Organization and date.	No. of cases.	Organization and date.	No. of cases.
Troop A	18	Troop B—Continued.		Troop E—Continued.		Troop F—Continued.	
August 1	1	September 18	1	September 5	1	September 16	1
August 17	1	September 26	2	September 6	1	September 17	1
August 19	1	September 28	1	September 11	1	September 18	2
August 20	3	Troop D	16	September 23	1	September 19	1
August 21	4	July 29	1	October 7	1	September 21	1
August 22	1	July 30	2	October 27	1	September 28	2
August 24	1	August 2	1	October 29	1	September 30	1
August 26	2	August 6	2	Troop F	29	October 1	1
August 27	1	August 10	1	July 29	1	October 20	1
September 1	1	August 18	1	July 30	1	Troop H	3
September 4	1	August 19	2	August 1	1	September 8	1
September 23	1	August 22	1	August 2	1	September 15	1
Troop B	12	August 23	1	August 29	2	September 23	1
August 1	1	October 1	1	August 30	2	Band	3
August 2	1	October 2	1	September 1	3	August 2	1
August 18	2	October 22	1	September 3	1	August 14	1
August 24	1	October 23	1	September 4	2	October 15	1
September 6	1	Troop E	9	September 5	1		
September 12	1			September 6	2		
September 16	1	September 3	2	September 7	1	Total	90

Table showing the strength of each company, the number of cases of dengue fever, and the percentage infected in the Sixteenth and Thirteenth Regiments of Infantry.

Name of organization.	Sixteenth U. S. Infantry.			Thirteenth U. S. Infantry.		
	Strength.	No. of cases.	Percentage infected.	Strength.	No. of cases.	Percentage infected.
Band	35	3	8	36	8	22
Company A	50	20	40	62	4	6
Company B	52	26	50	62	19	30
Company C	50	25	50	58	20	34
Company D	54	24	44	55	27	49
Company E	56	1	1	60	16	26
Company F	57	3	5	60	7	11
Company G	51	7	13	54	8	14
Company H	59	8	13	60	35	48
Company I	50	12	24	50	23	46
Company K				58	35	60
Company L	51	24	48	57	27	47
Company M	48	9	18	55	11	20

Table showing the strength of each company, the number of cases of dengue fever, and the percentage infected in the Eighth Cavalry.

Name of organization.	Eighth U. S. Cavalry.		
	Strength.	No. of cases.	Percentage infected.
Band	39	3	7
Troop A	56	18	32
Troop B	59	12	20
Troop D	56	16	28
Troop E	55	9	16
Troop F	55	29	52
Troop H	58	3	5

From the above tables it will be seen that the greatest ratio of infections in any one company was in Company H of the Thirteenth Infantry where 58 per cent of the men were infected, while in Company E of the Sixteenth Infantry only one man was infected, but this company left the post during the early days of the epidemic and thus can not be considered in this relation. It should also be remembered that Companies A, B, C, D of the Thirteenth and E, F, G, H of the Sixteenth were absent from the post after August 12, 1906.

In considering this table it will be noticed that the epidemic of dengue began in the Sixteenth Infantry upon July 1, reached the Thirteenth Infantry July 7, and the Eighth Cavalry not until July 29. It should be stated that the barracks of the Eighth Cavalry are at a considerable distance, at least 1 mile, from those of the Sixteenth Infantry, which, if we consider the disease to be mosquito-borne, will account for the long period of time elapsing between the infection of the two organizations.

On August 1, 1906, the First Battalion of the Thirteenth Infantry and the Second Battalion of the Sixteenth were ordered for field service in Leyte, P. I., and were still absent on this duty on November 1, 1906. At the time the First Battalion of the Thirteenth Infantry left Fort McKinley, 58 men had been sent to the post hospital suffering from dengue. No cases developed in this battalion on its voyage by boat to Leyte, nor have any cases developed in the battalion in Leyte. If dengue were a contagious disease this certainly would not have been the case, but removal from the focus of infection and from the disseminator of the infection—that is, the mosquito—resulted in a complete disappearance of dengue from this battalion.

At the time the Second Battalion of the Sixteenth Infantry left Fort McKinley, on August 12, dengue had just appeared, this battalion

only having sent about 20 cases to the post hospital suffering from the disease; three days before leaving it sent 5 more, but no cases developed upon the voyage to Leyte, nor have any cases appeared in this battalion since arriving in Leyte. It seems to us that the almost instant disappearance of dengue from these battalions upon their removal from Fort McKinley and their voyage by sea to Leyte is almost positive proof of the relation of the mosquito to the transmission of the disease. It is obvious that no mosquitoes were present upon the boat, and that, as probably no cases were infected previously to embarking, the mosquitoes in Leyte did not become infected, and therefore dengue did not continue in these troops.

Another fact of importance in considering the transmission of this disease is the manner in which it spread from barrack to barrack. If the disease were purely contagious we would expect contiguous barracks to become infected in order. As a matter of fact, as shown by the map, in which the barracks are numbered in the order in which they became infected, contiguous barracks did not always become infected in the order one would suppose; thus, of two barracks standing side by side, one might be infected two or three weeks before the other, whereas another several hundred yards distant from the first might be the second one to become infected. A careful study of the map will show that this is true in a great many instances, and it can be explained, we think, by the erratic flight of an infected insect, such as the mosquito.

Lack of contagion is strongly suggested by the fact that although over 400 cases of dengue were treated in the general medical wards of the post hospital, and although no special means were employed to prevent contagion, no cases developed in the wards except in three attendants who were on night duty.

In our own camp, where the patients under our observation were treated, we have had 128 cases. Here no person developed the disease, except after inoculation and in consequence thereof, though attendants and patients mingled freely, and we made every effort, as will be seen in the detailed report of cases, to convey the disease by fomites, or air. We had dengue patients and well men sleep together, eat together and wear one another's clothing; both sick and well meanwhile abstained from bathing, and used a common close-stool kept in the tent with them. All the sick recovered and none of the well men developed the disease throughout the experiments, unless they were inoculated later.

We therefore feel justified in stating that *dengue is not contagious*, and think that the history and epidemiology, taken in connection with Graham's work and what we later detail, justify the correlative statement that *the disease is mosquito-borne.*

III. ETIOLOGY.

1. HISTORICAL.

The etiological factor concerned in the causation of dengue has been long and patiently sought for by almost every investigator who has studied the disease; thus, nearly every fluid, secretion and excretion of the body has been examined, and, as might be expected, not a few observers have announced, from time to time, the discovery of a causative organism. Many of these so-called "discoveries" were made during the dawn of bacteriological science, and a perusal of the methods adopted in isolating the organisms described, as well as the description of their morphology is sufficient to prove their absolute lack of scientific accuracy. Therefore, we have not deemed it necessary to review such contributions, but among the many who have investigated the etiology of this disease there are a few whose observations demand consideration. Among these may be mentioned McLaughlin, Graham, Carpenter and Sutton, Guiteras and Agramonte.

The early investigations regarding the etiology of dengue were directed toward a bacterial cause, but within the past few years the increasing importance of the protozoa in the etiology of disease has turned the current of investigation, and almost all of the work which has been done upon this subject by recent investigators has been founded upon the belief that the disease is caused by some protozoön, probably by one inhabiting the blood.

However, despite the fact that a great amount of labor has been expended in the search for a bacterial or protozoal organism, it is surprising how few records of this work there are and, upon analysis, how unsatisfactory the conclusions arrived at. Perhaps, in no other disease, as common and as thoroughly studied clinically as dengue, is there so limited a literature concerning its parasitic etiology and it must be admitted that this is one of the infections of man which, up to the present, has baffled all attempts at a solution of its etiology.

The earliest attempt, based upon bacteriological methods, to discover the organism causing dengue was that of McLaughlin (23). The descriptions of his experiments are detailed and the results obtained are of interest, but, viewed in the light of the approved bacteriological methods of to-day, are open to very severe criticism and have not been confirmed by late observers.

McLaughlin, whose observations were published in 1886, believed in the directly contagious character of the disease and that organisms of a bacterial nature, existed in the blood of the infected individual. He examined both fresh and stained specimens of blood, and cultures from blood made upon nutrient gelatine. He also made microscopical examinations of the vomitus and urine and endeavored to secure cultures of the organism by partially filling sterilized glass bulbs with blood from a vein and allowing the blood so obtained to incubate for weeks and even months. By this method he examined but one case, but by the methods previously given he studied forty. In the blood of every case he found spherical micrococci measuring one-twentieth to one-thirtieth the diameter of a red blood corpuscle, and red or purplish in color; in preparations made from cultures grown upon nutrient gelatine, the cocci, when in masses, appeared black or brown, but when seen singly the red color was always distinct and characteristic. In the bulbs mentioned, which contained only blood (no bouillon), the same organisms were found in pure culture after an incubation period of from six weeks to three months.

While the researches of McLaughlin appear to have been partially accepted, or at least considered seriously by some writers, we regard them as only of purely historical interest, for reasons which are obvious.

Klein investigated very carefully the report of the presence of a short bacillus in the blood of dengue cases and concluded that there was not sufficient evidence to prove the constant association of this or any other organism with the disease. Wright was unable to demonstrate any organism as concerned in the etiology of dengue, and similar results followed the investigations of Crookshank and MacFadyen.

The observations of Graham (21) of Beirut, published in 1903, awakened increased interest in the etiology of this puzzling disease, and a considerable amount of work has since been done with the object of confirming or disproving his results. Graham's investigations were carried out in Beirut, Syria, and resulted in the announcement by him that dengue is due to a protozoön inhabiting the red blood corpuscles and closely resembling the plasmodia of malaria, except for the absence of pigment. He examined the blood of over one hundred cases, but in his communication regarding the subject he does not state in how many of these he found the parasite, but admits that he was unable to demonstrate it in stained smears of the blood. As described by Graham, the parasite first appears as a small, hyaline rod or dot within the red blood corpuscle, constantly changing its position, the motility in some instances being very marked; later, the parasite increases in size, appears to present typical amœboid motion, and finally almost fills the red corpuscle, or rupturing it becomes free in the plasma, where it very soon degenerates. No sporulating forms are described, nor any other method of reproduction in man. The organism is never observed to contain any pigment. He states that he has found the same bodies in the blood of patients suffering from second and third attacks of the disease.

From the results which this investigator obtained in transmission experiments to be mentioned later, he believed that the organism described underwent a developmental stage within the mosquito, and he therefore endeavored to trace such a cycle, using mosquitoes of the genus *Culex fatigans* Wied. for the purpose. By dissecting insects which had bitten dengue patients and examining the blood contained in the stomach, he claims to have demonstrated his piroplasma-like bodies in the mosquito up to the fifth day after the biting, and states that they undergo developmental changes similar to those occurring in the blood corpuscles in man. He did not succeed in finding any *zygotes* or any evidence of sexual forms within the mosquito. He also claims to have observed the spores of this organism "in among the cells of the salivary glands" after forty-eight hours in mosquitoes which have bitten a dengue patient upon the fourth day of the disease; he further claims that the spores could be demonstrated in the salivary glands of mosquitoes which had been kept a month.

Graham produced a very severe case of fever resembling dengue by inoculating a man subcutaneously with peptonized normal salt solution containing the salivary glands of a mosquito which had bitten a dengue patient twenty-four hours before. He was deterred from further experimentation along this line by reason of the very severe symptoms produced in this case.

Because of the positive character of Graham's statements his work attracted widespread attention and encouraged many investigators to study with renewed energy the etiology of dengue, but although many experienced microscopists have endeavored to confirm his results none have done so as regards the presence of a parasite within the blood corpuscles. With the exception of Eberle, whose description of a *plasmœba* obviously applied to vacuoles, artefacts, etc., which are so common in the blood of fever patients, no investigator claims to have been able to demonstrate in dengue blood in nature, any constant parasite, bacterial or protozoäl.

Carpenter and Sutton (22) studied 200 cases of dengue upon the Isthmus of

Panama, and examined both fresh and stained specimens of blood, using Wright's, Ehrlich's, the Romanowsky and Nocht's methods, as well as bacterial stains. They were unable to demonstrate McLaughlin's micrococcus or any other microörganisms in the blood. They found the nonpigmented bodies of Graham in unstained specimens, but not in stained ones. They also found the same bodies in the blood in other diseases; and regarded them as being due to necrobiotic changes in the red cells. They also examined mosquitoes which had bitten dengue patients, but found no parasites present in the insects.

Guiteras (7), as the result of a very careful investigation in Habana in 1905, believes that Graham is mistaken regarding his organism and concludes, after examining a large series of blood specimens taken during all days of the disease, at various hours and stained by various methods, that the blood contains no structure resembling a parasite.

The investigations by the staff of the Government Laboratories (24) at Manila, P. I., in 1900 into the etiology of dengue, resulted negatively as to the presence of a parasite. Their conclusions were as follows: (1) In dengue fever there is no leucocytosis; (2) the differential count of the white corpuscles in this disease show normal proportions of the several varieties; (3) the hæmatozoön described by Graham has not been found present in the circulating blood of our cases; (4) the micrococcus described by McLaughlin has not been encountered.

Agramonte (19), studying the disease in Habana in 1906, was unable to demonstrate any parasite in the blood, and the recent researches of Kieweit de Jonge and de Haan (25), in Java, which were most thorough, were also without result. Stitt (20), working upon the subject in Cavite, P. I., was unable to demonstrate any organism in the blood.

Method of transmission.—Until the publication of Graham's work practically nothing had been done in the way of experimental research directed toward the discovery of the method of transmission of dengue fever. We have already touched upon the theories regarding this question in considering the epidemiology of the disease, but so far as we have been able to determine, after consulting all the literature available, to Graham belongs the credit of first attacking this problem in a practical and scientific manner. His work aroused much interest, and however he may have erred in his interpretation of the bodies described by him as the cause of the disease, we believe that his experiments regarding the method of transmission are most valuable and his conclusion that dengue is transmitted by the mosquito is well founded and has been experimentally confirmed.

Graham's experiments regarding mosquito transmission were briefly as follows: Four men in good health were selected and slept night after night beneath mosquito bars containing mosquitoes that had bitten dengue patients. In one case the disease developed four days from the date of the first exposure, in one in five days, and in one in six days. In one case the result was negative. During the time the experiment lasted the men remained in their homes, where there had been no other cases of the disease, and where none developed later. In order to obviate the possibility that these men might have contracted the disease in some other way, there being a very severe epidemic in the city at that time, Graham took mosquitoes that had bitten dengue patients to a village situated in the mountains, where no cases of the disease had occurred. At this village he liberated the mosquitoes under the nets of two young men, living in different localities, and orders were given that the men were not to leave the nets until permitted. One of these men developed a very severe attack of dengue in four days after exposure, the other in five days. The mosquitoes were destroyed and the men continued to sleep under the mosquito bars for some

time after recovery. No other cases of the disease occurred in this village. The mosquitoes used in these experiments are stated by Graham to be *Culex fatigans* Wied., and the insects were used within a short time after biting infected patients. Graham further states that in Beirut no *Anopheles* are to be found, but that during the dengue epidemic the city was infested with great numbers of *Culex fatigans* Wied. It will be seen that of six healthy men bitten by infected mosquitoes, five developed dengue, two in four days, two in five days, and one in six days.

Since Graham's experiments and results were published, a few observers have endeavored to confirm them, but without success. Carpenter and Sutton (22) experimented with various mosquitoes, viz: *Culex stimulans* Walk., *C. tarsalis* Coq., *Stegomyia fasciata* Fbr., etc., but not with *Culex fatigans* Wied. The mosquitoes used were young, well-grown insects, reared by them and kept for from five to fourteen days after biting, when they were dissected and very carefully examined. No protozoa were demonstrated, nor any other organism which they regarded as of etiologic importance. The authors regard their mosquito inoculation experiments as untrustworthy, and state that no definite statements can be made concerning them. They give but four experimental cases of mosquito inoculation, two of which were negative, one positive after six days and one after two weeks from the date of mosquito bites; the latter case is obviously of no value, as the incubation period is too long. The authors state that "the volunteer subjects were not only exposed to the bites of other moquitoes at all times but they were also brought into almost daily contact with dengue cases.

Agramonte (19), in Habana, attempted to transmit the disease by mosquitoes, trying various species at various intervals after the insects had fed upon dengue patients, but was unsuccessful in producing the disease in this way. He believes, however, that dengue is transmitted by some species of mosquito, and that the reason for failure in experimental infection lies in some undiscovered fault in technique. The epidemic he studied was accompanied by a plague of *Culex fatigans* Wied.

In Habana, Guiteras and Finlay (7) endeavored to transmit the disease with *Culex pipiens* Linn., but with negative results. Guiteras states, regarding these experiments, that their small number and lack of variety deprive the negative results of a claim to conclusive character, and that their faith remains unshaken that the mosquito is the transmitter of dengue.

It is rather surprising, in view of the scientific interest and importance of this question, that so little has been done to prove or disprove the results claimed by Graham from his mosquito experiments; it is not, however, surprising, that those who have attempted to solve the problem have met with so little success, for only those who have done so can realize the difficulties and disappointments which await the investigator in this particular field of research. In our work upon this phase of the subject, to be detailed later, the experimental results obtained, while to our own satisfaction proving that *Culex fatigans* Wied. transmits dengue fever, have been, at times, most disappointing and discouraging; unknown natural conditions appear to be necessary for the transmission of this disease by the mosquito, and because of our ignorance of the method of securing these we believe that experimental transmission by the mosquito is rendered most difficult and that negative results may be expected much more frequently than positive ones.

2. EXAMINATION OF THE BLOOD.

In attempting to solve the etiology of dengue and its method of transmission, our attention was first directed to the microscopical examination of the blood of patients suffering from the disease. We have already noted the failure of numerous observers to confirm the presence of McLaughlin's or Graham's organisms, and also their negative results as to other blood parasites. Despite these we considered that our work would be incomplete without careful examination of both fresh and stained preparations of the blood, and accordingly we have studied thoroughly, in this respect, a large number of our cases; the blood was examined during every period of the disease, but especially during the first two days and during the terminal rise in the temperature; various staining methods were used, including Wright's stain and the methods used in demonstrating *Treponema pallidum*. The latter methods were used very carefully and in numerous cases, as at the time we began our work we were greatly inclined to believe that the organism concerned in the etiology of dengue might belong to the spirochætæ. We have not been able to confirm the results of McLaughlin or Graham, nor have we been able to demonstrate any organism in the blood of dengue patients which we can consider as an etiological factor.

There is but little in the literature concerning the changes in the blood in this disease, and even Graham's claim that the disease is due to a hæmatozoön which destroys the red corpuscles does not seem to have stimulated research in this direction. We shall, therefore, in this portion of our report, detail the results of our study of the blood which demonstrate that, whatever the cause of dengue may be, it is not an organism that influences to any marked extent the essential characteristics of the blood, with the exception of the relative proportions of the various forms of leucocytes.

(*a*) *Hæmoglobin.*—In uncomplicated cases the hæmoglobin and color index are normal.

Erythrocytes.—Number: Dengue is not a disease in which anæmia is present. We have made numerous blood counts in severe cases, and have never observed a count lower than 4,500,000 red cells per cubic millimeter, even when the count was made at the termination of the disease. This fact alone appears to us conclusively to disprove the existence of Graham's hæmatozoön, which by destroying the red blood corpuscles during its development within them would certainly reduce their number. We have never seen a case of uncomplicated dengue in which the clinical symptoms suggested anæmia. Our observations are borne out by those of Carpenter and Sutton (22), who found that the red blood count in dengue was generally over 5,000,000 per cubic millimeter.

Morphology: In size the red blood corpuscles are unchanged. Poikilocytosis is not commonly observed, but in some cases, during the height of the fever, a moderate degree of poikilocytosis may be present. Crena-

tion does not occur more rapidly nor is it more marked in dengue than in other acute, febrile conditions. Vacuolation is common both in fresh and stained specimens of blood, and in many instances the shape and appearance of the vacuoles is very suggestive of a parasitic invasion of the red cell; artefacts, due to degeneration of the protoplasm and clear areas due to retraction of the hæmoglobin, are common, especially in poorly prepared smears and are well calculated to lead to error because of their resemblance to bacterial or protozoal organisms. We have not observed that the appearance of the vacuoles occurring in the red corpuscles in dengue differs from that seen in many other febrile conditions, but it is certainly true that they frequently present an appearance very suggestive of amœboid motion without change of position; the progressive motion referred to by some writers which we have observed in the case of rod-shaped artefacts, is probably due to protoplasmic currents within the degenerating red cell.

It is not uncommon to observe in the blood of dengue, as well as in that of other febrile conditions, cocci or bacilli, either free in the blood plasma or attached to the red blood corpuscles; in the vast majority of instances these bacteria are due to external contamination and have no relation to the disease in which they are observed; when they are attached to the red blood cell and still possess some motility their resemblance to a parasite is often striking, but it is possible by gentle pressure to dislodge them and thus demonstrate their real nature.

We have not observed the presence of normoblasts or megaloblasts in the blood in dengue, and their absence, especially of normoblasts, indicates that anæmia even of a mild type, is not present.

The staining reactions of the red corpuscles in dengue do not differ from those present in health. Polychromatophilia or basophilia we have not observed, but in poorly prepared specimens the staining may be irregular, suggesting granular degeneration.

From our observations we conclude that the morphology of the red cell in dengue shows no diagnostic changes.

(*c*) *The leucocytes.*—Number: One of the most important blood changes in this disease is the presence in almost every case of a marked leucopenia. From our observations we are convinced that the leucopenia of dengue is almost constant throughout the attack, and that it is of considerable diagnostic importance. We have made leucocyte counts in a large number of cases and have invariably found a marked reduction in the total number with, as will be seen later, a quite characteristic change in the relative proportion of the various forms. The lowest leucocyte count was 1,200 per cubic millimeter, the highest 4,860, the average, 3,800. Carpenter, and Sutton (22) found a constant leucopenia, the lowest count being 1,866, the highest 5,866, the average about 3,500, per cubic millimeter. Stitt (20) states that a leucopenia is always present; and his counts varied from 1,450 to 5,280 per cubic millimeter. We have found

that the leucopenia is progressive, being most marked upon the fifth or sixth day of the disease.

Morphology: We have observed no morphological changes in the leucocytes, nor any evidence of the presence of a leucocytozoön.

Differential blood count: From the studies of Carpenter and Sutton, and later of Stitt, the differential leucocyte count in dengue has assumed considerable diagnostic importance, and taken together with the leucopenia appears to us to be entitled to very careful consideration in the differential diagnosis of dengue, yellow fever, malaria, and the eruptive fevers.

Carpenter and Sutton (22), from their examinations, conclude that in dengue there is always a leucopenia, and generally an increase in the small lymphocytes and in the eosinophiles, the latter occurring late in the disease.

Stitt (20) made differential leucocyte counts at varying periods of the disease. He found that a marked variation occurred in the different forms of the leucocytes at different periods, there being at first a large increase in the small lymphocytes, succeeded by a greater increase in the large lymphocytes, and finally, during the terminal eruption, a most marked increase in the large mononuclears.

Because of lack of time we have made comparatively few differential leucocyte counts, but our results have been supplemented by those of Lieutenant Vedder, Medical Department, United States Army, stationed at Fort William McKinley, who kindly volunteered to assist us in this direction, and whose tables and remarks are given below. From our own observations we are loath to lay much stress upon the variation in the relative proportion of the large and small lymphocytes, as does Stitt, for in many instances we have not found a constant relationship between the variety of lymphocyte which is increased and the period of the disease, but we have found a constant leucopenia, a decrease in the polymorphonuclears and an increase in small lymphocytes. In one of our experimental cases (Case 9) in whom we produced a severe attack of dengue by the intravenous inoculation of the filtered blood from another experimental case, the leucocyte counts made upon the first, third and sixth days of the disease, well illustrate the changes described by Stitt, as will be seen by the following record:

First day of disease:	
Polymorphonuclears	50
Small lymphocytes	41
Large lymphocytes	7.5
Eosinophiles	1.5
Third day of disease:	
Polymorphonuclears	52
Small lymphocytes	36
Large lymphocytes	8
Eosinophiles	4
Sixth day of disease:	
Polymorphonuclears	48
Small lymphocytes	14
Large lymphocytes	32
Eosinophiles	6

It will be observed that the eosinophiles showed a marked increase as the disease progressed, and this has been noticed in several of our cases. While the above differential count is typical of the results obtained by some observers, we have found, even in a limited number of examinations, that it is not of sufficiently frequent occurrence to be depended upon alone in reaching a diagnosis. In fact, in most of our counts we found that the small lymphocytes outnumbered the large in every stage of the disease.

Vedder, as a result of his observations upon the white blood count in dengue, has submitted to us the following data. His blood counts were made on patients suffering from the disease occurring in the same epidemic which we have described above. Regarding his results he says:

> The polymorphonuclear leucocytes are greatly decreased and the small lymphocytes are greatly increased, while the large lymphocytes are moderately increased during the latter days of the illness. These changes are, to a greater or less degree, characteristic of the disease throughout its duration. The decrease in polymorphonuclears and the increase in small lymphocytes takes place almost at once, being very noticeable on the second day of the illness.

Vedder regards the differential leucocyte count as of great value in the diagnosis between this disease and yellow fever.

The following table gives in percentages, the results obtained by Vedder in five cases in which a differential count was made on each day of the disease:

Day.	Leucocytes.	Polymorphonuclears.	Small lymphocytes.	Large lymphocytes.	Mononuclears.	Eosinophiles.
CASE 1.						
	Number.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	5,300	79.50	16.00	2.50	0.50	1.50
2	4,100	50.00	43.00	5.50	1.50	0
3	5,900	45.00	48.25	5.75	1.00	0
4	4,400	46.00	40.25	10.75	3.00	0
5	4,700	58.25	34.00	6.30	1.25	0
6	5,250	46.33	43.33	8.66	1.00	.66
7	4,700	29.50	56.00	11.75	2.50	0
8	4,800	27.50	60.00	11.25	.50	.75
CASE 2.						
1	4,200	60.00	17.75	7.50	2.00	12.75
2	3,800	40.50	45.00	10.00	3.00	1.50
3	6,500	25.00	54.33	8.00	2.00	10.66
4	2,750	34.00	44.00	6.00	1.00	15.00
5	2,600	45.00	45.66	7.66	1.00	1.66
6	4,550	23.33	48.33	8.33	.66	19.33
7		38.75	50.00	8.50	1.75	.50
8	5,550	30.00	47.33	6.33	.66	15.33
9		30.00	46.66	11.66	1.33	10.00

Day.	Leucocytes.	Polymorphonuclears.	Small lymphocytes.	Large lymphocytes.	Mononoclears.	Eosinophiles.
CASE 3.						
	Number.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent*
1		67.50	25.25	6.24	1.00	0
2		50.66	43.33	4.66	1.00	.33
3		29.00	59.25	8.25	2.50	.25
4		27.00	52.00	16.00	2.50	1.50
5		30.00	55.00	10.00	2.00	3.00
6						
7		37.00	36.50	14.00	9.50	3.00
CASE 4.						
1		62.00	29.50	7.00	1.00	0.50
2		48.00	45.66	4.66	1.00	.33
3		54.50	37.00	5.00	2.50	1.00
4		56.50	33.50	3.50	1.00	5.50
5		38.00	50.00	8.00	1.00	3.00
6		34.50	47.00	11.50	2.00	5.00
7		41.00	36.75	11.25	3.50	7.25
8		28.66	43.33	12.66	3.33	2.33
CASE 5.						
1		75.33	16.66	6.00	0.33	1.33
2		45.66	46.00	5.33	3.00	0
3		48.75	45.25	4.50	1.00	.50
4		37.00	51.00	8.00	2.00	1.00
5		29.00	58.50	8.50	3.50	0
6		27.00	47.50	18.75	3.75	2.25
7		35.25	46.00	13.75	1.50	3.25

The following table gives the average of ten differential counts made on each day of the disease for eight days:

Table showing average of ten counts made on each day of disease for eight days.

Count.	Polymorphonuclears.	Small lymphocytes.	Large lymphocytes.	Mononoclears.	Eosinophiles.
FIRST DAY.					
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	79.50	16.00	2.50	0.50	1.50
2	85.25	10.75	2.75	1.00	.25
3	60.00	17.75	7.50	2.00	12.75
4	78.00	17.33	3.00	1.33	.33
5	58.00	36.33	4.00	1.66	0
6	55.66	37.66	6.00	.66	0
7	68.75	22.00	6.75	2.25	.25
8	66.25	26.25	5.25	1.00	.25
9	72.75	23.00	3.00	1.25	0
10	67.50	25.25	6.25	1.00	0
Average	69.16	23.28	4.70	1.26	1.53

Table showing average of ten counts made on each day of disease for eight days—Cont'd.

Count.	Polymorphonuclears.	Small lymphocytes.	Large lymphocytes.	Mononoclears.	Eosinophiles.
SECOND DAY.					
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	42.80	42.60	6.80	1.00	6.80
2	47.50	46.50	5.50	.50	0
3	27.50	65.75	5.00	.75	.25
4	60.00	43.00	5.50	1.50	0
5	49.00	44.00	6.00	.50	.50
6	40.50	45.00	10.00	3.00	1.50
7	48.00	39.00	9.00	3.00	1.00
8	32.66	59.00	6.00	2.33	0
9	50.66	43.33	4.66	1.00	.33
10	48.00	45.66	4.66	1.00	.33
Average	43.66	47.38	6.31	1.46	1.07
THIRD DAY.					
1	58.00	34.50	6.50	1.00	0
2	48.75	45.25	4.50	1.00	.50
3	54.50	37.00	5.00	2.50	1.00
4	29.00	59.25	8.25	2.50	.25
5	35.33	47.33	11.66	3.00	2.66
6	33.50	46.00	13.50	6.00	.50
7	51.00	33.66	12.66	2.00	.66
8	37.00	50.66	10.33	2.00	0
9	29.00	58.33	10.66	2.00	1.00
10	25.00	54.33	8.00	2.00	10.66
Average	40.10	46.63	9.10	2.40	1.72
FOURTH DAY.					
1	38.75	54.75	5.50	0.25	0.50
2	46.66	34.33	8.66	5.33	4.33
3	59.75	33.00	2.50	.25	4.25
4	34.66	57.33	5.66	.66	1.00
5	46.00	40.25	10.75	3.00	0
6	34.00	44.00	6.00	1.00	15.00
7	36.66	55.00	6.66	1.00	.66
8	27.00	52.00	16.00	2.50	1.50
9	32.25	54.75	10.00	3.00	.25
10	37.00	51.00	8.00	2.00	1.00
Average	39.27	47.64	7.97	1.89	2.84
FIFTH DAY.					
1	25.66	47.66	14.33	0.66	11.00
2	34.00	42.33	18.66	2.66	2.00
3	38.00	50.00	8.66	1.66	.33
4	59.75	33.00	2.50	.25	4.25
5	58.25	34.00	6.50	1.25	0
6	45.00	45.66	7.66	1.00	1.66
7	30.00	55.00	10.00	2.00	3.00
8	38.00	50.00	8.00	1.00	3.00
9	29.00	58.50	8.50	3.50	0
10	38.00	46.60	10.80	3.00	1.40
Average	39.56	46.27	9.56	1.69	2.66

Table showing average of ten counts made on each day of disease for eight days—Cont'd.

Count.	Polymorphonuclears.	Small lymphocytes.	Large lymphocytes.	Mononoclears.	Eosinophiles.
SIXTH DAY.					
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	17.60	70.60	10.00	1.80	0.80
2	33.00	49.00	12.75	3.75	1.00
3	45.75	41.75	8.50	1.25	2.50
4	55.00	35.00	7.40	.60	1.60
5	46.33	43.33	8.66	1.00	.66
6	23.33	48.33	8.33	.66	19.33
7	29.33	57.33	11.33	1.33	.66
8	34.50	47.00	11.50	2.00	5.00
9	34.00	56.33	7.66	1.00	.66
10	52.33	34.33	9.66	1.00	2.66
Average	37.11	48.30	9.57	1.43	3.48
SEVENTH DAY.					
1	29.50	56.00	11.75	2.50	0
2	38.75	50.00	8.50	1.75	.50
3	37.00	36.50	14.00	9.50	3.00
4	41.00	36.75	11.25	3.50	7.25
5	35.25	46.00	13.75	1.50	3.25
6	41.50	52.00	4.50	.75	1.25
7	31.75	60.75	6.75	.75	0
8	25.00	57.75	12.50	3.50	1.00
9	17.50	64.75	12.00	1.50	4.00
10	51.66	40.66	5.66	1.00	1.00
Average	34.89	50.11	10.01	2.62	2.12
EIGHTH DAY.					
1	27.50	60.00	11.25	0.50	0.75
2	44.00	35.66	14.33	2.66	3.00
3	30.00	47.33	6.33	.66	15.33
4	38.33	47.33	6.66	2.66	4.66
5	28.66	43.33	21.66	3.33	2.33
6	41.75	47.50	8.50	.50	1.50
7	42.33	37.33	13.33	3.66	2.00
8	48.66	35.66	11.66	.66	3.33
9	51.33	36.00	5.33	0	6.66
10	37.50	53.50	5.50	.75	2.50
Average	39.00	44.36	10.45	1.53	4.20

Summary.—From our examinations of the blood in dengue we consider that the following conclusions are justified:

1. There does not occur in the blood of dengue any visible organism, either bacterial or protozoal in nature, which can be considered as the cause of the disease. We have not observed any protozoön in the blood.

2. Dengue is not accompanied by anæmia, the red blood count being normal in uncomplicated cases. There are no characteristic morphological changes in the red corpuscles, leucocytes, blood plates or blood plasma.

3. Dengue is characterized by a leucopenia and in the vast majority of instances by a decrease in the polymorphonuclear leucocytes and a marked increase in the small lymphocytes; the increase in the small lymphocytes is constant throughout the disease.

(*d*) *Blood plates.*—We have observed no changes in the number or appearance of the blood plates in dengue.

(*e*) *The blood plasma.*—In neither fresh nor stained specimens of blood have we been able to demonstrate any organism of etiological significance in the blood plasma in dengue. In a few instances bacteria were noticed but from the ease with which blood cultures collected with the most careful attention and asepsis become contaminated in this climate, we believe that these bacteria were of external origin. The most common bacterium observed was a long, stout bacillus, actively motile, and commonly seen here in blood specimens from various sources. No organism resembling a protozoön was observed in the blood plasma. Yeast cells were frequent contaminations in stained specimens.

3. BLOOD CULTURES.

With the exception of McLaughlin's researches, which have been mentioned and which, so far as cultural methods are concerned, were almost valueless, we have not been able to find in the literature any detailed descriptions of experiments having for their object the cultivation of bacteria or protozoa from the blood of dengue patients. We therefore approached this portion of our task with high hopes that by applying modern cultural methods we might be able to isolate and cultivate the organism causing the disease. In view of the success attained by Novy and others in the cultivation of trypanosomes, and by Rodgers in cultivating the Leishman-Donovan body (*Herpetomonas donovani*), we were especially hopeful that by applying similar methods in this disease, we might be able to secure growths of any protozoön which might be present. As we have stated, we were impressed with the idea that dengue might be caused by an organism belonging to the same group as those producing relapsing fever, or to some closely allied group, and we therefore endeavored, both by employing special staining methods and culture media, to demonstrate such an organism.

(*a*) *Methods.*—In our experiments we have used citrated blood and acid and alkaline bouillon as culture media. In making cultures with citrated blood, the sterilized syringe was first filled with citrated solu-

tion, which was then ejected, a very little being allowed to remain in the needle; the syringe was then filled with blood by plunging the needle into a prominent vein of the forearm and withdrawing the blood very slowly until the barrel of the syringe was full; the blood was then ejected into small sterilized glass tubes and kept at room temperature in the incubator or in the lower compartment of an ice box, the latter in order to give any organism undergoing a part of its life cycle in a cold-blooded animal, surroundings congenial to its development.

In making blood cultures in bouillon, 10 cubic centimeters of blood obtained from the median basilic vein were added to 250 cubic centimeters of bouillon contained in 500 cubic centimeter flasks, and incubated at temperatures of from 26° to 36.05° C.

In preparing our cultures the utmost care was taken to avoid infection, everything being rendered sterile that could be, but despite all of our precautions the majority of our cultures sooner or later became contaminated with various forms of bacteria or yeasts.

(*b*) *Citrated blood cultures.*—In eight cases we have endeavored to secure cultures of the organism causing dengue by citrating blood obtained from dengue patients at various periods of the disease. In none of these have we been able to demonstrate any organism which we can consider to be of any etiologic significance; in none of the tubes of citrated blood did we encounter any organism resembling, in the least, a protozoön, and all of the bacteria observed were evidently contaminations. A small diplococcus occurred in two cases, but in the light of our later work on filtered blood this obviously is of no significance.

(*c*) *Bouillon blood cultures.*—In twelve cases we used bouillon blood cultures, allowing them to incubate for as long as eight weeks. The majority of the flasks became infected, but in four cases the blood cultures did not show any growth at the end of eight weeks, when they were destroyed. A staphylococcus grew in one in forty-eight hours, a diplococcus in three in seventy-two hours, accompanied by a large spore-bearing bacillus in two of the cases; a short, thick, motile bacillus, together with a staphylococcus in one, in four days and various spore-bearing baccilli in the remainder. These organisms we believed to be contaminations and therefore we did not perform any experimental work with them. Our conclusions regarding them were confirmed by the result of our experiments with filtered blood

As the result of our culture experiments we were forced to conclude that no organism was found constantly enough in the cultures to warrant us in regarding it as having an etiological relationship to the disease, especially as a number of them remained sterile, although kept for as long as eight weeks.

INOCULATION EXPERIMENTS.

Having thus failed to demonstrate any organism in either fresh or stained specimens of blood or in our blood cultures, we directed our attention to the possibility of producing the disease by the inoculation of blood from the dengue patient into the healthy man; fortunately for the success of our work we were dealing with a disease which, in the young and robust, is not dangerous to life, and for this reason we felt justified in making such experiments. We hoped in this way to determine the presence or absence of the infective agent in the blood, for should such experiments prove successful they would demonstrate that the cause of the disease is in the blood, and that therefore, insect transmission, is possible, whereas negative results would prove that the blood does not contain the organism unless it be one that first has to undergo a developmental cycle outside of the body, as in an insect, before it can produce the disease in man.

In order to secure subjects for experiment a call for volunteers was issued to members of the Hospital Corps serving at the United States Army Division Hospital, and four men volunteered, in all of whom we succeeded in producing dengue by the intravenous inoculation of blood from cases of the disease. We desire to express our admiration of the courage and devotion to duty of these men, who, with no prospect of pecuniary reward, cheerfully placed themselves in our hands for experimentation.

As more men were needed and as no more Hospital Corps men were available, we consulted Major General Leonard Wood, United States Army, commanding the Philippines Division, who authorized us to offer a reward for volunteering, as a result of which we secured many more volunteers than we needed and were forced to refuse a large number, as we were limited to sixteen, including the men we had already used. Unfortunately, of the fourteen men we have experimented upon, seven came from Fort William McKinley having passed unharmed through the dengue epidemic, and of these men we found two absolutely immune, three relatively immune, and one doubtful. Of the same number of Hospital Corps men who had not been exposed to dengue, we found only one immune.

Of the fourteen soldiers who volunteered for this work, seven belonged to the United States Army Hospital Corps, three to the Eighth United States Calvary, two to the Sixteenth United States Infantry, one to the Thirteenth United States Infantry, and one to Company B., Engineer Corps.

4. INTRAVENOUS INOCULATION WITH UNFILTERED DENGUE BLOOD.

Eleven of our fourteen volunteers were given intravenous inoculations of unfiltered blood from dengue patients, and of these, seven developed the disease, while in one case the result was doubtful. In three of the cases there existed, apparently, an absolute immunity to the disease. These cases will now be considered in detail.

Experiment No. 1.

Case 1, Chart 1.—E. U., private, Hospital Corps, United States Army. Had not been exposed to dengue. At 3.30 p. m., July 24, 1906, he was given an intravenous injection of 20 minims of unfiltered blood from Case 20 (see Chart A). The patient from whom the blood was taken for the inoculation had a mild attack of dengue and was probably nearly over the disease at this time. We believe that this accounts for the mild character of the experimental disease in this case, for while the symptoms which were present were typical, it will be seen on referring to the temperature chart that the fever was slight as compared with our other experimental cases, which were all inoculated with blood from more severe cases, taken at an earlier period of the disease. The following is a résumé of the clinical record in this case:

July 24: At 3.30 p. m. inoculated intravenously with 20 minims of blood from Case 20. Subject in good health and temperature normal.

July 25: Feels perfectly well.

July 26: Feels well. No symptoms.

July 27: Ditto.

July 28: Has some fever and headache. Bowels constipated.

July 29: Patient feels uncomfortable, complaining of vague muscular pain and burning and smarting of eyes; eats and sleeps fairly well. Bowels regular.

July 30: Last night felt very uncomfortable, having severe headache and pain in eyes; also pain in the lumbar muscles, ankles, elbows and wrists. At present (11 a. m.) complains of dull headache and slight lumbar pain; the eyes are painful, the pain being aggravated by movement. Tongue moist and clean. Bowels constipated.

July 31: Feels much better, the pains in the head and muscles having disappeared. A slight eruption is present upon the chest and back.

August 2: Feels well. Eruption has faded.

August 6: Returned to duty.

Remarks.—Upon reference to the temperature chart (Chart 1) it will be observed that the temperature began to ascend about 9 a. m. on July 28, but the patient complained of no symptoms until nearly twenty-four hours later. The incubation period in this case, therefore, is about three days and eighteen hours, reckoning it from the 9 a. m. temperature on July 28.

Experiment No. 2.

Case 2, Chart 2.—W. R. H., private, first class, Hospital Corps. Not previously exposed to dengue so far as known. In good health. At 11 a. m., July 31, 1906, was given an intravenous inoculation of 20 minims of blood from Case 30 (Chart B), who was suffering from a typical attack of dengue of four days' duration at the time the blood was obtained. No symptoms appeared in Case 2 until early in the morning of August 3, as is shown by the following résumé of the clinical record:

July 31: Inoculated with dengue blood as stated.

August 1 and 2: Feels well.

August 3: At 2 a. m. patient awakened by pain in chest and some difficulty in breathing. This soon passed away and patient slept until morning. Felt well upon awaking this morning but soon developed sharp pain in the head, muscles of the back and legs. Complains also of a slight cough and pain in the eyes. Had slight chill at 5.30 p. m. to-day. No appetite.

August 4: Patient complains of severe pains in the lumbar region but has no headache. Diarrhœa is present, the stool being watery in character. No appetite. His general appearence is typical of dengue.

August 5: Still complains of the lumbar pain; at 9 p. m. complained of abdominal pain, accompanied by slight nausea, vomiting and diarrhœa. No eruption has been observed.

August 6: Patient feels well.

August 7: Patient feels well.

August 8: Has headache and pain in muscles and joints. Last night was very nervous, almost delirious. Otherwise feels well.

August 9: Feeling well. Returned to duty August 14, 1906.

Remarks.—In this case the incubation period, as shown by the accompanying chart, was approximately two days and nineteen hours. The temperature curve is typical of a moderately severe case of dengue and the symptoms corresponded; the terminal rise and fall is well shown in this chart. No eruption occurred at any time, although otherwise the symptoms were typical, with the exception of the diarrhœa, which was present for a short time, accompanied by nausea and vomiting, which we regard as rather the exception than the rule in dengue.

Experiment No. 3.

Case 3, Chart 3.—E. W., private, first class, Hospital Corps, United States Army. At the time of inoculation this man was perfectly well and had not been exposed to dengue. He was given an intravenous inoculation at 2 p. m., August 16, of 20 minims of blood from Case 36 (see Chart C). The latter case was typical of dengue, which, at the time the blood was taken had lasted a little over three days. The clinical record of Case 3 follows:

August 16: Inoculated with dengue blood at 2 p. m. to-day, the injection being made into a vein of the forearm.

August 17 and 18: Patient feels well.

August 19: Slight temperature last night, but no headache, pain or other symptoms.

August 20: Last night patient suffered from headache, pain in bones and muscles of back, and severe pain in the knee articulations. Slept poorly. This morning has headache, located in the temporal region, and general muscular pain. Eyeballs are tender to the touch and also pain. Stomach and bowels normal. No appetite. An eruption, which appeared this morning covers almost the entire body, but is especially marked over the trunk and arms; it consists of very fine, slightly elevated papules, dark-red in color, the color disappearing upon pressure.

August 21: Feels somewhat better this morning. Has pain in eyes, shoulders, wrists and knees. The eruption is still present but is not so vivid in coloring as yesterday. Appetite poor. Bowels regular. Tongue clean and moist.

August 23: Patient had more headache last night but feels much better this morning. Is having very little pain now. The eruption is still present and the itching is constant. The bowels are loose. Appetite good.

August 24: Had headache last night which caused insomnia, also aching pain, severe in character, in muscles and joints. The eruption has greatly in-

creased, covering the entire body, being especially marked over the trunk and limbs.

August 25: Patient slept well and all symptoms have disappeared except slight headache and pain in the eyes. The eruption is fading and the itching is very annoying; there is considerable desquamation present in the form of small white scales.

August 27: Eruption has disappeared. Patient feels well.

August 31: Returned to duty.

Remarks.—The incubation period in this case was about two days and eighteen hours. The temperature curve is not as characteristic as is generally observed, but the symptoms were very typical, and the fact that we were able to produce a very severe case of dengue by the injection of the filtered blood of this patient proves beyond doubt the nature of the disease (see Case 9, Chart 9). An interesting feature of this case is the early appearance of a well-marked eruption, which, after fading gradually, increased again during the crisis and finally disappeared, accompanied by considerable desquamation. It is also interesting to note that in the case of dengue referred to as being produced by the inoculation of filtered blood from this case, the eruption appeared early and presented the same characteristics. While the temperature was low the symptoms were more severe than in many others in which the fever was much greater, and the eruption was almost as well marked as in any case that we have observed.

In the three cases just described the inoculations were made as soon as the subject volunteered, no previous experiment in the way of exposure to fomites or mosquitoes having been tried. In the case which follows, the inoculations were used as a final test of immunity, the men inoculated having been exposed to both fomites and infected mosquitoes.

Experiment No. 4.

Case 4, Chart 4.—C. H. B., private, Troop A, Eighth Cavalry. Had been exposed to dengue, his troop having had eighteen men on sick report with the disease. At time of volunteering he was in good health, and stated that he had never had any serious illness. The following is a brief résumé of the clinical record in this case:

September 22: Exposed to fomites of dengue, being placed with three dengue cases in a mosquito-proof tent, sleeping in their beds and wearing their underclothes.

September 26: No result being obtained from fomites after four days, the patient is to sleep to-night under net with about forty mosquitoes which bit Case 38, who had a typical case of dengue (see Chart D) on the night of September 25.

September 27: Feels well. Says he was not bitten by a single mosquito.

September 28: Feels well. Says that he has not yet been bitten by the mosquitoes.

September 28 to October 2: During this time the patient has slept under the net containing the infected mosquitoes, but claims that he has not been bitten at all, and his statement is confirmed by the fact that the mosquitoes all remained empty during this time, and made no attempt to bite, most of them dying while within the mosquito net. He states that so far as he knows he has never been bitten by a mosquito, although he had campaigned in localities in Africa which were almost uninhabitable because of these insects. At first we were inclined to doubt his statements in this respect, but observation has convinced us that this man is really immune to mosquitoes.

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October 3: At 10 a. m. the patient was given intravenously 20 minims of blood from Case 44 (see Chart E), a very typical case of dengue of about three and one-half days' duration.

October 4 and 5: Feels well.

October 6: Last night about 10.30 the patient complained of fever and muscular pains. This morning has severe pain in head, back and limbs. Face greatly flushed, conjunctivæ congested. Bowels constipated. Tongue moist with thin white coating.

October 7: Vomited last night. Has less pain this morning, located mostly in the muscles of the loins and thighs. Has considerable headache and pain in the eyes upon movement. Tongue moist and entirely covered with a yellowish coat. Bowels normal. Feels much better but still has muscular pain and headache.

October 9: Feeling well.

October 10: Had headache and pain last night, but feels well this morning.

October 15: Returned to duty.

Remarks.—The temperature curve in this case is one often observed in naturally acquired dengue and the symptoms throughout were typical of the disease. The absence of an eruption is to be noted, not that it is unusual in many natural infections, but because in our experimental cases an eruption was almost always present. Thus of the ten cases in which we were successful beyond doubt in producing dengue, eight presented well marked eruptions, while one was somewhat doubtful in this respect.

Experiment No. 5.

Case 5, Chart 5.—C. R. D., second-class private, Company B, Engineer Corps, United States Army. This man at the time of volunteering was in good health, but had been exposed to dengue during the Fort William McKinley epidemic. He was observed for a period of four days before any experiments were made. The following is the clinical record of this case:

September 19: At 1 p. m. a small abrasion was made on the mucous membrane of his cheek, and the patient then rinsed his mouth with blood from a dengue case, diluted with salt solution. No results were obtained from this experiment.

October 4: Patient slept last night under a mosquito-bar with mosquitoes that had bitten a typical case of dengue the night before. He was bitten several times during the next few nights, but dengue did not develop.

October 22: At 3 p. m. to-day an intravenous inoculation of 20 minims of blood from a dengue case (Case 60, Chart F) was given the subject.

October 23, 24, and 25: Patient is feeling well.

October 26: At 7 p. m. to-day the patient complained of headache, pain in the lumbar region and the legs, and loss of appetite.

October 27 and 28: Patient complains of severe headache, pain in the lumbar region and in the joints. His hands and wrists are slightly swollen, and the face, arms and hands greatly flushed. He is constipated and has no appetite.

October 29: Feeling much better. An eruption has appeared upon the chest and abdomen, resembling more the eruptions due to heat than a dengue eruption. He is still constipated and has but little appetite.

October 30 and 31: Patient feels well. There is a marked rash over the back and chest which upon the 31st had extended to the arms and legs. This rash is a typical dengue rash.

November 1: The rash is still well marked and is present over the entire body, including the palms of the hands and the soles of the feet.

November 2: Patient feeling well and the eruption has almost disappeared.

November 5: Returned to duty.

Remarks.—It will be observed that this patient did not contract dengue through the mucous membrane of the mouth nor from mosquito bites. As regards the latter experiment, we can not be sure that he was bitten more than one or two times by mosquitoes. From the intravenous inoculation of dengue blood he developed a very typical case of the disease as will be seen by referring to the temperature chart. The incubation period in this case was longer than in any of our previous cases, being four days and four hours.

Experiment No. 6.

Case 6, Chart 6.—J. E. S., private, Troop H, Eighth Cavalry. At time of experiments this man was in good health, but had been exposed to dengue at Fort McKinley. The following is a record of the experiments performed in this case:

September 22: Exposed last night to mosquitoes that bit Case 41 (Chart G) on September 13. Says that none bit him during the night.

September 23: Exposed again last night to mosquitoes, and says he was bitten once. Dengue did not result from this experiment.

October 7: Patient was exposed to mosquitoes that bit Case 44 (Chart E) the night before.

October 8 to 15: Patient feeling well, and states that he does not know whether he has been bitten. Dengue did not result from this experiment.

October 25: Slept last night with mosquitoes that had bitten Case 60 (Chart F), and was bitten at least twice.

October 26 to 28: Mosquitoes have disappeared and patient has not been bitten again. Dengue did not result from this experiment.

October 31: Slept with mosquitoes that had bitten Case 65 (Chart J) last night.

November 1 to 3: Has been bitten several times by the mosquitoes.

November 4: Patient feeling well and mosquitoes have all disappeared. Dengue did not result from this experiment.

November 8: At 3 p. m. to-day the patient was inoculated intravenously with 20 minims of blood from Case 70 (Chart K). The man from whom the blood was taken had a typical attack of dengue fever, and the inoculation was made upon the third day of the disease.

November 10 to 14: Patient feeling well.

November 15: In the afternoon the patient had a slight chill, headache and general pains in the muscles.

November 16: Patient complains of severe headache, backache and pain in the limbs. Has no appetite.

November 17 to 20: During this time the patient presented the usual symptoms of dengue, which have already been indicated.

Remarks.—The chief point of interest in this case is the long period of incubation, exceeding that of any experimental case that we have observed. Inoculation was made at 3 p. m. on November 8, and the first symptoms appeared in the afternoon of November 15, making the incubation period about seven days. It should be remembered that this man had already passed through a severe epidemic of dengue, and it is probable that he possessed a relative immunity to the disease, although his clinical symptoms were typical and rather severe in character.

Experiment No. 7.

Case 7, Chart 7.—W. J., private, Troop D, Eighth Cavalry. This case is of interest because the experimental dengue was complicated by an attack of malarial fever. He had been exposed to dengue at Fort McKinley but was in good health at the time of volunteering. The following is the clinical record of this case:

September 19 to 26: Patient was exposed to fomites of dengue during this time in the usual manner.

September 26: Exposed to mosquitoes that had bitten Case 80 (Chart L) the night before. This case was afterward found to be suffering from æstivo-autumnal malaria.

September 27 to October 8: Patient is feeling well.

October 8: Slept last night under a bar with mosquitoes that bit Case 4 (Chart 4), and upon the night of the 9th was bitten twice. Dengue did not result from this experiment.

October 25: Slept last night under a bar with mosquitoes that bit Case 81 (Chart M) the night before. Patient states that he did not feel well during the night, and complains of pain in the back of the head and in the lumbar region.

October 26 to October 28: Patient is feeling better. æstivo-autumnal malarial parasites were found in his blood on the afternoon of October 27, and quinine at once administered.

October 30 to November 4: Patient is feeling well. No further experiments were instituted until November 17.

November 17: Inoculated at 10.30 a. m. with 20 minims of blood from Case 82 (Chart N), who was suffering from a typical attack of dengue, which had lasted about three days.

November 18 to November 23: Patient feeling well.

November 24: At 10 a. m. patient had a slight chill, followed by high fever. Upon November 22 an eruption had been noticed covering the entire body, which resembled the eruption of dengue.

November 26: Patient is feeling well, and is free from pain. He states that he had had considerable pain for several days before his chill. He is covered with an abundant rash which presents all the characteristics observed in our other dengue cases.

Remarks.—There are several points of interest in this case. Upon September 26 he was exposed to the bites of mosquitoes that had bitten a case of æstivo-autumnal malaria. Upon October 25 he developed an attack of malarial fever, and the æstivo-autumnal parasites were found in his blood. He denied that he had ever suffered from malaria previously, and while we have been careful to liberate only mosquitoes of the genus *Culex fatigans* Wied., beneath the bars of the men experimented upon, it may be that an *Anopheles* may have been present with the mosquitoes liberated. However, as the *Anopheles* mosquitoes are present here, it is much more probable that the infection occurred from mosquitoes which had bitten a malarial case at some other time. It is difficult in this case to determine just exactly the period of incubation, and the chart is also atypical because of the concurrent malarial infection. However, the clinical symptoms were very typical, and the presence of the rash removes all doubt as to the nature of the infection.

Experiment No. 8.

Case 8, Chart 8.—R. R., private, Company H, Thirteenth Infantry. This man was in good health at the time of volunteering, but had been exposed to dengue during the epidemic at Fort William McKinley. The following is a clinical record of the case:

September 12: Inoculated intravenously with one-half minim of blood from Case 83 (Chart O), who was suffering from a typical attack of dengue.

September 15: Patient states that he had a severe headache last night, commencing at midnight. Still complains of headache and pain in the arms.

September 16: Still complains of headache, but has no other pain. Bowels constipated, appetite poor.

September 17 to 19: Patient is feeling well.

September 19: Inoculated intravenously at 1 p. m. with 1 cubic centimeter of filtered blood from Case 11 (Chart 11). No result.

September 25: Inoculated intravenously at 1 p. m. with 20 minims of blood from Case 38 (Chart D). No result.

Remarks.—We have regarded this case as doubtful, although we are inclined to believe that the rise of temperature upon September 15 was due to a slight attack of dengue produced by the inoculation of one-half minim of dengue blood. This is much less blood than we have used in our other experiments and it may be that the slight symptoms produced are due to this fact. If this man did not suffer from an attack of dengue during his first inoculation he must have been immune, as neither the subcutaneous inoculation of filtered blood or the intravenous inoculation of unfiltered blood produced any result.

Summary.—The intravenous inoculation of unfiltered dengue blood into healthy men is capable of producing a typical dengue attack in such men. Thus, of eleven men so inoculated, seven suffered from dengue fever produced in this manner, while in one the result was doubtful. Three of the men were immune to the disease after inoculation.

5. INTRAVENOUS INOCULATION OF FILTERED DENGUE BLOOD.

Having proven by our inoculation experiments with unfiltered blood from dengue patients that the disease could be thus transmitted and, furthermore, that while the cause must, therefore, be present in the blood, it is not possible to demonstrate it in either fresh or stained specimens by any known method of examination, we are forced to the conclusion that the causative organism must be ultramicroscopic, as in the case of yellow fever, rinderpest, etc.

The most important diseases which have been proven to be due to a contagion which passes through porcelain or diatomaceous filters are foot-and-mouth disease, pleuro-pneumonia of cattle, yellow fever, rabies, rinderpest, South African horse sickness, and hog cholera.

Loeffler and Frosch (26), in 1898, discovered that the cause of foot-and-mouth disease in cattle passed through the pores of a Berkefeld filter which restrained other well-known bacteria; Nocard and Roux (27), in the same year, proved that the organism causing the pleuro-pneumonia of cattle passed through a Berkefeld and Chamberland F filter, but not through a Chamberland B; they were also successful in cultivating the organism by the collodion-sac method, but as they

demonstrated that it could be seen, though indistinctly, with a power of 2,000 diameters, it can hardly be claimed that this organism is ultramicroscopic. In 1902, Reed and Carroll (28) filtered the blood of yellow fever patients and proved that the virus passes through a Berkefeld filter by producing the disease by inoculating the filtrate; Rosenau and Francis (29), in 1903, found that the cause of yellow fever passes through a Chamberland B filter. The virus of rabies, according to Remlinger and Riffat Bey (30), passes through the most porous of the Berkefeld filters, but not through other porcelain or diatomaceous filters. In rinderpest, Nicolle and Adil Bey (31) have proven that the cause passes through the Berkefeld and Chamberland F filters; Nocard (32), in 1901, found that the virus of South African horse sickness passed through the Berkefeld filters, and in 1900, McFadyean (33) demonstrated that it also passed through the pores of a Chamberland F and Chamberland B filter. The recent investigations of Dorset, Bolton, and McBryde (34) prove that the organism causing hog cholera is filterable through Berkefeld, Chamberland F and Chamberland B filters, and as easily through one as the other; they also prove that the organism so long regarded as the cause of the disease—that is, *B. cholera suis*—is not concerned in the etiology of hog cholera, the disease being produced by the filterable virus. Of the diseases mentioned, the causative organism has been demonstrated, with the highest power of the microscope, in but one—that is, pleuro-pneumonia—and in this instance no morphological details could be distinguished, the organism appearing simply as a minute motile point. In all the others the parasites are ultramicroscopic.

In order to determine if dengue belonged to this class of infections, we determined to try the effect of the intravenous inoculation of filtered blood from dengue patients into healthy men. We have experimented in this way upon two men in both of whom we have been successful in producing very typical attacks of dengue accompanied by rather severe symptoms.

FILTERS USED AND CONTROL METHODS.

In our filtration experiments we have employed a Lilliput diatomaceous filter, which was tested each time before it was used. Before using, the filter was sterilized and the filtration done under 730 millimeters pressure.

After filtering the blood the following control test of the filter was made in each case: A suspension, in nutrient bouillon, was made of *M. melitensis* and *S. choleræ*, and then filtered through the filter used in filtering the blood; the filtrate was then incubated for two weeks, daily examinations of it being made. The filter in use retained both these organisms, the filtrate remaining sterile for two weeks when it was thrown away. In the control filtrations the same filter was used, after careful sterilization, as was employed for the dengue blood, and the same pressure was maintained during filtration.

Besides the control test of the filter, we kept in each case a portion of the filtered dengue blood for a period of ten days, making daily examinations, and in one case, several cultures in bouillon. No growth was obtained in either the filtered blood or the cultures.

Experiment No. 9.

Case 9, Chart 9.—E. J. D., private Hospital Corps, United States Army. On August 21, 1906, 10 cubic centimeters of blood was drawn from the median basilic vein of Case 2, an experimental case of dengue which has been described (see Case 2, Chart 2). The blood was taken on the third day of the disease, the symptoms of the patient at the time consisting of fever, headache, severe pain in the muscles of the shoulders, in the wrists and knees, while there was present a typical dengue eruption.

The blood was rapidly defibrinated, diluted with an equal amount of normal salt solution, and filtered through a Lilliput filter, controlled as described. The filtration of a sufficient quantity for use was completed in about three-quarters of an hour. Of the filtrate, 50 minims, containing 20 minims of the filtered blood, was inoculated intravenously into Case 9 at 4.20 p. m., August 21. The patient at the time of inoculation had been in the hospital under observation for several weeks, and as no cases of dengue had occurred in the hospital, he had not been exposed to the disease. No symptoms of importance developed until August 25, the period of incubation being three days and eleven hours. Previous to the decided onset of the disease there had been slight fever and some pain in the back, symptoms which were probably due to a gonorrhœa from which he had suffered for some time. The following is the clinical record of this case:

August 21: Inoculated intravenously at 4.20 p. m. with 25 minims of filtered dengue blood from Case 2.

August 22 and 23: Feeling well.

August 24: Slight muscular pains.

August 25, 9 a. m.: Patient complains of pain in muscles of neck, shoulders and knees. Has some headache. Bowels constipated. Tongue moist and clean.

August 25, 4 p. m.: The symptoms have increased in severity. The headache is frontal and intense; there is severe pain located behind the eyeballs, which are painful on pressure. There is general muscular pain, especially in the muscles of the jaw, lumbar region, and in the calves of the leg. Patient states that his bones ache and that he is unable to rest comfortably in any position. He also complains of severe pain in the articulations. An eruption is present, covering the chest, abdomen and thighs, and is especially marked over the forearms and around the wrists; it is dull red in color, consisting of minute elevations surrounded by a vivid flush, which makes the rash appear confluent in character.

August 26: Patient passed a very restless night, suffering from insomnia and severe pain in the back, chest, legs, head, and eyes. This morning still has severe pain in these regions. There has been no vomiting and the bowels are constipated. Tongue is moist, with a slight white coating. The eruption covers the entire body and is more marked than yesterday. There is complete loss of appetite and the patient is very restless.

August 27: Feels much more comfortable this morning. The steady ache in the muscles has disappeared but he still suffers from lancinating pains in the head, back, and legs. The eruption has almost disappeared.

August 28: Passed another restless night and suffered a great deal from pain in the muscles. This morning he complains of severe headache and pain in the loins and legs. The eruption has faded from the trunk. Has no appetite.

August 29: Feels better this morning. Still has headache and pain in the eyes, but the general muscular pain has disappeared.

August 30: Still complains of pain in the head and eyes but slept well last night.

August 31: Patient states that he feels very well this morning. Has no pain and appetite is returning. There is present a very profuse dengue eruption covering the entire body, especially marked upon the arms, legs, hands and feet.

September 1: Feels well. The eruption is less distinct although it still covers the entire body.

September 2: Is feeling well in every away and eruption has disappeared.

September 4: Discharged.

Remarks.—This case, as shown by the clinical record and the chart, is typical of severe dengue, but the initial eruption was more marked than in any of our cases. The patient suffered greatly from the headache and the muscular pains. He repeatedly stated that he felt as though every bone in his body had been broken.

The temperature chart presents a high range of fever, with not as marked a period of remission as is generally observed; it will be noted that morning remissions occurred regularly, but that in the afternoon the temperature ascended, reaching 104° F. on three consecutive days; a more permanent remission occurred upon the fifth day, succeeded upon the sixth by the final rise and the crisis, the temperature reaching normal upon the seventh day.

An eruption appeared in this case upon the first day (the so-called initial eruption), extending over the chest, abdomen and thighs. The typical dengue eruption occurred, as is usual, during the crisis, and was very profuse, extending over the entire body, even the hands and feet being covered with it. The severe initial eruption in this case is very unusual, and it is most interesting to find, upon reference to the clinical history of Case 2, from whom this man was inoculated, that an eruption occurred in this case also upon the second day of the disease.

Experiment No. 10.

Case 10, Chart 10.—B. S., first-class private, Hospital Corps, United States Army. On August 31, 1906, at 12.15 p. m., this man, who had been on duty at the Division Hospital for weeks and had not been exposed to dengue, was given an intravenous injection in the arm of 3.75 cubic centimeters of normal salt solution containing 20 minims of dengue blood from Case 87 (Chart H). Ten cubic centimeters of blood was taken from the medium basilic vein of Case 87 at 10.30 a. m., August 31, diluted with normal salt solution, and filtered through the same filters used in Case 9, the filter being controlled as has been described. This filtered blood was used for the inoculation. The patient from whom the blood was obtained was suffering from a rather severe attack of dengue, and the blood was taken on the fourth day of the disease.

After inoculation with the filtered blood no symptoms appeared in Case 10 until midnight of September 3, but upon referring to the temperate chart it will be noticed that he had fever at least sixteen hours before he complained of any symptoms. If we assume that the first rise in temperature indicated the onset of dengue, the incubation period must have been about two and one-half days, while if the chill, which was the first symptom the patient noticed, is considered as marking the onset, the incubation period would be just three days. We consider that the incubation period in this case was two and one-half days. The following is a résumé of the clinical record of this case:

August 31: Inoculated intravenously at 12.30 p. m. with 20 minims of filtered blood from Case 87.

September 1: Was restless last night but at noon to-day feels well.

September 2 and 3: Feeling well.

September 4: Had chill last night about midnight. This morning complains of pain in the muscles and bones, especially of the arms. His eyes ache and are

much congested and the face is flushed. Has slight frontal headache, and is very nervous. He complains of palpitation of the heart. Tongue moist and clean. Appetite poor.

September 5: Is feeling very nervous this morning and was delirious last night. Has pain in head, back, arms and legs. No appetite. Tongue moist, with heavy, yellowish coating. Bowels loose.

September 6: Spent a restless night, but is not so nervous this morning. Complains of severe pain in the back and legs. Tongue moist, with yellowish coating. There is a faint, slightly elevated, sparse, macular eruption over the chest and back.

September 7: Patient had a comfortable night and this morning has but little pain. The eruption is well marked over the abdomen, chest, back and arms.

September 8: Feeling very comfortable. The eruption is fading a little. Bowels constipated. Appetite good.

September 9: Was delirious during the early morning hours and is nervous and restless this morning, but free from pain. The eruption has largely disappeared.

September 10: Began to feel better at 4 p. m. yesterday and now feels quite well. Slept well, but perspired very freely during the night. The eruption has almost disappeared from the body, but is marked upon the forearms and wrists.

September 11: Feeling well. Eruption is fading slowly and very slight desquamation is present in patches.

September 13: Feeling well, except that appetite is still poor. The eruption has almost disappeared.

September 15: Returned to duty.

Remarks.—The symptoms in this case were very severe, especially those connected with the nervous system. The subject of the experiment was of a highly nervous temperament, and this fact accounts, in our opinion, for the severity of the nervous symptoms.

The temperature curve in this case might be used as an illustration of an ideal dengue curve, so perfectly does it agree with the type described by every observer as characteristic of this disease. It should be noted, however, that the temperature is higher in this case than it usually is in naturally acquired dengue, or in our other experimental cases, with the exception of Case 9, also produced by the intravenous inoculation of filtered blood.

The eruption in this case appeared on the fourth day of the disease, and had disappeared on the third day following the crisis, lasting in all ten days.

We regard these two cases of dengue produced by the intravenous injection of filtered dengue blood as the most typical cases of the severe type of the disease which we have observed and we believe that these two experiments prove conclusively that dengue can be transmitted by blood which has been passed through a filter which retains organisms as small as 0.4 μ in diameter (the measurement of *M. melitensis*). It also proves that in all probability the causative agent is ultramicroscopic in size, for the reason that neither in fresh nor stained blood smears nor in the filtrate obtained from dengue blood, can any organism be demonstrated with the microscope. It may be possible that in some other fluid or organ of the body, or in some phase of its life history in an insect, the organism may be visible, for Novy, in his work upon *T. lewisi*, has proved that even so large a parasite as this trypanosoma may exist in a form so

small in cultures that it passes through a Berkefeld filter. While this may prove to be true as regards the dengue organism, we feel justified in stating, that, so far as present evidence goes, the organism causing dengue is ultramicroscopic in size. This conclusion explains the uniformly negative results obtained by nearly every trained observer in the search for a dengue parasite.

We conclude that an organism is present in the filtrate, rather than a toxin, because of the length of the period intervening between inoculation and the appearance of clinical symptoms, and also because we have reproduced the disease by inoculation of the blood of experimental cases.

There is one point of interest deserving of special consideration in these two cases of dengue produced by filtered blood; that is, the relatively greater severity of the symptoms. In both these cases the symptoms were more intense in almost every particular than in any of our experimental cases, despite the fact that no greater amount of blood was inoculated in these cases. This fact is very difficult of explanation, and we must confess to our ignorance of the cause. It may be that the admixture with salt solution or the time consumed in filtration, or both, acts in some way to increase the virulence of the organism, or that conditions favorable to its extra-corporeal development are present during the process of preparing the filtrate which result in a more virulent form of the organism, though we have no evidence to offer in this respect.

6. EXPERIMENTAL TRANSMISSION OF DENGUE BY THE MOSQUITO.

We have already mentioned the experiments of Graham (21), regarding the transmission of dengue by the mosquito, in which he seems to have proven conclusively that such transmission occurs; we have also noted the negative results obtained by Carpenter and Sutton (22), Guiteras and Cartaya (7), and Agramonte (19), all of whom believe, however, that the mosquito is the active agent in the spread of the disease. To one who carefully studies the epidemiology of dengue, the conclusion is almost inevitable that this disease, which so closely resembles yellow fever and malaria in this respect, must also be transmitted by some species of mosquito. Its seasonal prevalence; its occurrence most frequently along low-lying, moist, coast regions and in the valleys of rivers; its rapid diffusion in certain localities, and its lack of diffusion in others; its relation to changes in temperature and moisture; its manner of spread from building to building in infected places; the presence of multitudes of mosquitoes wherever dengue occurs, and the absence of the disease in regions where mosquitoes are few in number or absent, and the cessation of the epidemic in badly infected districts when conditions arise unfavorable to the propagation of mosquitoes, all point to some species of this insect as the transmitting agent.

Accordingly, having demonstrated by the intravenous inoculation of unfiltered dengue blood that the cause of the disease is present in the

blood of the infected individual, and that the parasite is probably ultramicroscopic in size, as proven by the positive results of our experiments with the filtered blood, we turned our attention to the problem of mosquito transmission. Unfortunately for the fullest success of our work in this direction, we were forced because of lack of other volunteers, to use a number of men who had already passed unharmed through the epidemic at Fort McKinley and the majority of whom were immune, as is proven by the negative result of intravenous inoculation of dengue blood. Thus, of the nine men in whom we endeavored to produce dengue by exposing them to the bites of infected mosquitoes, three were proven in this way to be absolutely immune, one may have had a slight attack of dengue previous to exposure, while three probably possessed a relative immunity, for while they developed dengue from the inoculation of a comparatively large amount of dengue blood, the symptoms were mild in character, and in one case the incubation period was greatly prolonged. In one instance already described (see Case 4) no immunity existed to the disease, but the mosquitoes refused to bite the man under any conditions we could devise.

The mosquito used.—In looking over the geographical distribution of dengue and various species of mosquitoes, we found but one species (*Culex fatigans* Wied.,) of this insect that apparently occurred wherever dengue did. We do not wish to be understood as stating conclusively that this mosquito is the only one which may be present in all dengue-infected regions, but only that, so far as we have been able to determine from the literature available, this species is constantly found and is mentioned by almost every recent investigator as being very numerous during epidemics of this disease. In Theobald's monograph the map illustrating the known distribution of *Culex fatigans* Wied. might almost be used to illustrate the distribution of dengue fever, and if to this map be added the regions in which this mosquito has been demonstrated since it was published, the association of dengue and *Culex fatigans* Wied., is still more striking.

For this reason, and because this mosquito was employed by Graham in his experiments, we decided to work with this species at first, and in the event of our results being negative, to extend our work to embrace other species.

We have used mosquitoes reared in captivity, and also those caught in natural surroundings. However, in our successful case produced by the mosquito, we used mosquitoes reared by us from the egg, and thus we are sure that no infection occurred in these insects before they bit the dengue patients.

Our mosquito experiments were conducted as follows: The patient suffering from dengue was placed in a bed beneath a mosquito net in a mosquito-proof tent. At night from twenty to thirty mosquitoes were liberated beneath the mosquito bar and collected in the morning; almost

invariably all the mosquitoes left alive had bitten and were full of blood. The subject to be experimented upon, having been placed in bed beneath a mosquito net in another mosquito-proof tent, the mosquitoes which had bitten the dengue case the night before were liberated beneath his mosquito net, and orders given that the man remain beneath the net until the mosquitoes had disappeared; later we allowed the men to remain out of bed during the day, the mosquitoes being kept beneath the spread net. With one exception, which has been noted, all the men were bitten a few times, but in most instances the mosquitoes died before the men had been bitten severely. We also confined mosquitoes that had bitten dengue cases in glass jars, and kept them as long as from four to six days before allowing them to bite, but in the few instances in which we tried this method our results were all negative.

We do not consider it necessary to give our negative results in full, as they are all referred to in detailed experiments, and we will only describe the case in which we produced dengue by allowing the volunteer to be bitten by infected mosquitoes.

Experiment No. 11.

Case 11, Chart 11.—B. L. W., private, Hospital Corps, United States Army. This man had been on duty at the Division Hospital for several weeks, and as no cases of dengue had occurred in the hospital, had not been exposed to the disease, so far as we could determine. On September 12, 1906, the man being in good health, he was placed under a mosquito net with mosquitoes that had bitten Case 88 (Chart R) on the night of September 11. Case 88 was suffering at the time from a typical attack of dengue. Case 11 was not bitten by mosquitoes until the night of September 13, and developed no symptoms until the night of the 17th, but upon reference to his chart it will be seen that he had fever for nearly twenty-four hours before he noticed any symptoms. If we assume the period of incubation to be the period intervening between the 13th, the night upon which he was first bitten, and the 16th, when he had his first rise in temperature, the incubation period would be about three days and one-half. However, if we assume the disease to have commenced when he first noticed symptoms—that is, upon the evening of the 17th—the incubation period would be a little over four days. The following is a summary of his clinical history:

September 12: Put under net with mosquitoes that bit Case 88 last night.

September 13: Bitten by mosquitoes last night.

September 18: Had headache and felt uncomfortable last evening. This morning complains of headache and a dull pain in the articulations.

September 19: Still complains of headache and general muscular pain and soreness. His face and eyes are greatly congested.

September 20: Last night had severe pain in the head, eyes and the muscles of the back, but feels much better this morning.

September 21: Is feeling better. A faint rash is visible covering the chest and abdomen.

September 22: Complains of soreness and stiffness in the muscles. The eruption is now plainly visible and typical of dengue.

September 24: Feels well. The eruption has almost disappeared.

October 1: Returned to duty.

Remarks.—This case was in every way typical of a moderately severe attack of dengue. The symptoms were those seen in the great majority of naturally acquired infections and the temperature chart is a very characteristic one. This man had not been exposed in our dengue camp before being bitten by the mosquitoes, and did not leave the mosquito-proof camp until after the onset of the disease.

For reasons which have been stated, of the nine men exposed to the bites of infected mosquitoes, only four can be considered in estimating the results obtained. Of these, one, or 25 per cent, developed a typical attack of dengue following the bites of infected mosquitoes; but we do not consider that the three negative cases are of much value, as the conditions were such as to cause some doubt as to whether the men were bitten.

It is obvious that many factors have to be considered in considering mosquito experiments, and it is more than probable that in our negative experiments we were unsuccessful in reproducing the favorable conditions which must have been present in Experiment No. 11, or the mosquitoes, if they became infected, may have perished before biting again. Schaudinn has recently called attention to some of the difficulties which may be met with in attempting the experimental transmission of a disease by mosquitoes. Thus, as he has shown, certain individuals of a species which has been proven to transmit a certain disease are not able to transmit it, and this may be due to the insect itself suffering from some other infection, to an inability to digest the ingested blood, to an acquired or natural immunity resulting in the death of the specific parasite, or the mosquito may die before it has bitten again.

It is evident from the result of Experiment No. 11 that the parasite causing dengue does not undergo any cycle of development within the mosquito, unless it be a very short one; we are, therefore, of the belief that the parasite of dengue is one capable of living in the stomach of the mosquito for an unknown period of time, where it retains its virulence; that infection may occur at any time after the insect has ingested blood containing the parasite, and that it is introduced into man when the insect bites, being regurgitated through the œsophagus and proboscis with the fluid from the stomach. This theory is borne out by the results recently obtained by the Indian Plague Commission in its remarkable study of the transmission of plague from rat to rat by the flea, *Pulex cheopis* Rothsch., and by the excessive rapidity of the diffusion of dengue, which would be impossible were the parasite one which underwent a prolonged cycle of development in the tissues of the mosquito. We have dissected and examined a large number of mosquitoes that had bitten dengue patients, but have never found any organism either in the stomach or tissues suggestive of a stage in the life cycle of a protozoön. We can not confirm Graham's results in this respect, and we believe that in the mosquito as well as in the blood of man, the dengue parasite is ultramicroscopic in size.

By reason of lack of suitable volunteers and the subsidence of the epidemic we have been forced to bring our mosquito experiments to a conclusion. We have been unable to investigate many interesting questions regarding the transmission of dengue by the mosquito, such as the length of time the insect remains capable of transferring the infection, the most infective period of the disease as regards transmission in this way, and whether transmission is simply mechanical or depends upon the development or multiplication of the parasite within the mosquito; all of these questions are of great importance to a correct conception of the etiology of dengue and there would appear to be no good reason why, in regions where the disease is common, they should not be thoroughly investigated. We realize that the work we have been able to do as regards mosquito transmission is very incomplete and that much remains to be done before this feature of the etiology of dengue is fully elucidated, but we believe that we have confirmed Graham's results in this respect and that we have proven experimentally that this disease may be transmitted by the mosquito *Culex fatigans* Wied. We also believe that mosquito transmission is the only natural method which has been proved by experiment and that all the epidemiological data confirm such a method of transmission.

7. EXPERIMENTAL PERIOD OF INCUBATION IN DENGUE.

As will be seen from a résumé of the epidemiology of dengue, the incubation period has been stated as varying from twenty-four hours to ten days, the majority of observers regarding it to be from three to five days. The following table gives the period of incubation in nine of our experimental cases of the diseases:

No. of case.	How produced.	Incubation period.
1	Inoculation of unfiltered blood	3 days 18 hours.
2	do	2 days 19 hours.
3	do	2 days 18 hours.
4	do	2 days 12 hours.
5	do	4 days 4 hours.
6	do	7 days
9	Inoculation of filtered blood	3 days 11 hours.
10	do	2 days 12 hours.
11	By mosquito	About 3 days 16 hours.

From the above table it will be seen that the incubation period of dengue in experimental cases of the disease varied from two and one-half days to seven days, the average being about three days and fourteen hours. This is practically the period of incubation stated as being most frequent by clinicians. We have observed no case in which the incubation period was as short as twenty-four hours, and from our experiments we very much doubt the occurrence of such a short period of incubation.

8. IMMUNITY AND SUSCEPTIBILITY.

There is considerable confusion existing in regard to these points, the general trend of opinion being that almost everybody is susceptible, and that an attack of dengue produces immunity for a short time only. As to the latter point—that is, the duration of acquired immunity—we can not express a very positive opinion, as we endeavored, except in one case, in our experiments to avoid the use of men who had previously had dengue. In the one case noted as an exception, dengue was induced although the patient said he had experienced three attacks, the last one two and a half years ago. We have also known a few other cases in which the disease developed naturally after a like period. The correctness of reports of cases in which attacks have occurred a month apart we very much doubt. We had about six patients sent back to us after such periods supposed to be suffering from second attacks, but in no case was it so. The "second attack" was usually a malarial paroxysm.

As to natural immunity, we know that it occurs, or at all events that it may be temporarily present. We think it altogether probable that it may be relative; that is, a small dose of virus may not be sufficient to overcome it, but a large one may. In one of our cases (Case 8) we were unable to decide positively whether an immunity which was present at the time of the discharge of the patient was natural or was acquired from a very light attack of the disease following inoculation with a half minim of blood, though we incline to the latter belief. In at least one instance immunity was apparent and not real; that is, the patient did not develop dengue when exposed to mosquitoes that had recently bitten other dengue cases, but this was due to the fact that the subject was immune to mosquito bites. All the mosquitoes put in his net died after periods varying from one to five or six days, and not one of them bit him. Later, when he developed dengue from the intravenous injection of blood, mosquitoes bit him freely. Fortunately, this characteristic so valuable in the Tropics, was not permanently lost, for the patient now states that he is as free from mosquito bites as before.

Our knowledge as to natural immunity cost us rather dear, as we were paying all our subjects of experiment, and did not relish exhausting the funds at our disposal in payments to men not capable of developing the disease. In the light of subsequent events we think that we made a mistake in accepting volunteers from Fort McKinley, where an epidemic of dengue had been and was prevailing; because, while we did not begin experiments upon the men until they had been under our observation and free from exposure to dengue for periods varying from a week to three weeks, and thus avoided the error of thinking the disease due to our inoculations when it was in reality due to other causes, we picked men, some of whom had probably escaped natural infection because of their natural immunity.

As we have stated, three of our subjects were absolutely immune to dengue. Our assumption, that failure to develop the disease after inoculation with 20 minims of blood from a dengue case constitutes absolute immunity, is arbitrary, but seems justified by the constancy and severity of the symptoms produced in the successful cases.

Three of the men possibly showed a relative immunity; that is, the amount of virus transferred to them by mosquitoes was not sufficient to cause the disease, although the intravenous injection of 20 minims of dengue blood was sufficient to do so. Possibly this relative immunity was only apparent, because we know that these men were not severely bitten by the mosquitoes, and we do not know that the particular mosquitoes that did bite them might not have been laboring under some disability that prevented their transmitting the disease. It is noteworthy that two of these cases were very mild, and that the third, while an ordinary one, presented an incubation period longer than the average.

Six cases, and if we count the doubtful one already described, seven, presented no immunity; that is, they developed dengue following the first attempt at inoculation. One case, immune to mosquito bites, showed apparent immunity, but developed dengue after the first inoculation.

Natural immunity and the practice of sleeping under mosquito nets effectually protected a large proportion of healthy men against infection. Thus, in the Fort McKinley epidemic, the highest percentage of infections occurring in any one company was 58, the next highest was 52, and in the other companies it was lower. It must be remembered that in this epidemic no special measures were adopted to prevent the spread of the disease, and the mosquito protection consisted merely of the ordinary routine use of nets during the sleeping hours.

IMMUNITY AS SHOWN BY EXPERIMENTS.

The following cases whose clinical records are here given were proved by experiment to be absolutely immune to dengue. The temperature charts are not reproduced, as they contain no data of interest.

Case 12.—W. H. O., first-class private, Hospital Corps, United States Army. This man was on duty at the Division Hospital at the time of experiments, and had never had dengue.

Experiment 1: On the night of September 12 the subject slept under a mosquito net with mosquitoes that had bitten a dengue case the night before. No symptoms of dengue developed.

Experiment 2: On the night of September 28 the subject was again exposed to mosquitoes that had bitten a dengue patient the night before. He was bitten repeatedly during the next few nights, but no symptoms of dengue developed.

Experiment 3: On October 3 the subject was inoculated intravenously with unfiltered dengue blood from Case 44 (Chart E). No symptoms of dengue developed, and the man was returned to duty October 11, 1906.

Case 13.—J. G., private, Company I, Sixteenth Infantry. This soldier belonged to a company of the Sixteenth Infantry that had furnished twelve cases to the hospital with dengue before this man volunteered. He had therefore been exposed to the disease.

Experiment 1: On September 12, 1906, the subject rinsed his mouth with normal salt solution containing 12 minims of dengue blood, our object being to determine if the dengue parasite could infect through an intact mucous membrance. The result of the experiment was negative.

Experiment 2: On September 19 the subject was given an intravenous inoculation of filtered blood from Case 11 (Chart 11). No symptoms developed.

Experiment 3: On the night of October 4 the subject was exposed to mosquitoes that had bitten Case 44 (Chart E) the night before, and was bitten at least twice. Dengue did not develop.

Experiment 4: On the night of October 15 he was bitten many times by mosquitoes that had bitten a dengue case two nights before. The result was negative.

Experiment 5: On October 22 the subject was given an intravenous inoculation of 20 minims of unfiltered blood from Case 95 (Chart S). No symptoms of dengue developed, and the man was returned to duty October 29, 1906.

Case 14.—J. B. P., private, Company M, Sixteenth Infantry. At the time of volunteering the company to which this man belonged had furnished ten men suffering from dengue to the Forth McKinley Hospital.

Experiment 1: On the night of September 24, 1906, the subject was exposed to mosquitoes that had bitten Case 11 (Chart 11) the night before. He was bitten several times and also many times during the next ten days. The result of the experiment was negative.

Experiment 2: The subject was exposed October 26 and 27 to mosquitoes that had bitten a typical case of dengue on October 25. The result of the experiment was negative.

Experiment 3: On November 17 the subject was given an intravenous inoculation of unfiltered blood from Case 82 (Chart N), who was suffering from a typical attack of dengue. No successful result was obtained in this experiment, and the man was returned to duty November 23, 1906.

Remarks.—These men were all exposed to fomites, in addition to the experiments outlined, and we believe that the results of these experiments demonstrate that absolute immunity to dengue is present in certain individuals.

9. CONTAGION IN DENGUE.

We have already noted the theories regarding the contagious character of dengue. We have carefully studied this portion of our subject, and believe that the following facts conclusively prove that dengue is not contagious in the least degree.

1. At the hospital at Fort William McKinley over 600 cases of dengue were treated in the general wards without a single case originating among the other patients in the wards. Only four men belonging to the Hospital Corps on duty at this hospital contracted the disease, three of them being nurses on night duty in the wards and the other a cook having no contact with the dengue patients. No precautions were used to prevent contagion other than the rigid use of mosquito nets at night, the dengue and other patients eating together, and being closely associated

throughout the day. It is noteworthy that the only men unprotected by the mosquito nets at night—that is, the three night nurses—all developed the disease.

2. In our dengue hospital, where we treated over 120 cases, no instance of infection occurred among the attendants, although their association with the dengue patients was very intimate and continued for over four months.

3. Our experiments with fomites were all negative. We endeavored to produce the disease by exposure of healthy men to fomites, the men experimented with living in mosquito-proof tents with patients suffering from dengue, throughout the entire course of the disease. They slept in their beds, wore their underclothing and pajamas, and ate and drank from the same table furniture. In this way we experimented with eight men, none of whom developed the disease from such exposure.

We conclude, therefore, that dengue is not a contagious disease, and that patients suffering from it may be placed in the general wards of a hospital without fear of infection, provided precautions are taken to protect the patients from mosquitoes.

Conclusions regarding the etiology of dengue.—From our study of the etiology of dengue, we believe the following conclusions are justified:

1. No organism, either bacterium or protozoön, can be demonstrated in either fresh or stained specimens of dengue blood with the microscope.

2. The red blood count in dengue is normal.

3. There occur no characteristic morphological changes in the red or white blood corpuscles in this disease.

4. Dengue is characterized by a well-marked leucopenia, the polymorphonuclear leucocytes being decreased, as a rule, while there is a marked increase in the small lymphocytes.

5. The intravenous inoculation of unfiltered dengue blood into healthy men is followed by a typical attack of the disease.

6. The intravenous inoculation of filtered dengue blood into healthy men is followed by a typical attack of the disease.

7. The cause of the disease is, therefore, probably ultramicroscopic.

8. Dengue can be transmitted by the mosquito, *Culex fatigans* Wied., and this is probably the most common method of transmission.

9. No organism of etiological significance occurred in bouillon or citrated blood cultures.

10. The period of incubation in experimental dengue averages three days and fourteen hours.

11. Certain individuals are absolutely immune to dengue, as proven by our experiments.

12. Dengue is not a contagious disease, but is infectious in the same manner as is yellow fever and malaria.

IV. SYMPTOMATOLOGY.

It is of cardinal importance in considering the symptoms and diagnosis of dengue to bear in mind the fact that it presents, in different epidemics and in different individuals in the same epidemic, a variety of clinical pictures; and that, while there is what may be called typical dengue, there are many variations from the type, and there is no one symptom that can be said to be pathognomonic, or even constant, if we except fever. We do not state positively that even fever is constant, but we are unable to satisfy ourselves that a given case is dengue unless it shows some fever, particularly at the onset. This doubtless accounts for the different descriptions of the disease that have been written. We agree with Guiteras and Cartaya in the belief that many cases can not be properly diagnosed except in the presence of an epidemic. We likewise agree with them that it is illogical to differentiate subtypes of the disease according to the dominant symptom, so we shall content ourselves with outlining the typical attack, and commenting on the usual symptoms. In doing this we will use the plan of the writers mentioned, whose observations and descriptions we consider accurate, clear and well balanced.

Invasion.—This is usually rather sudden, and, exceptionally in our experience, may be so sudden that the patient has to sit or lie down, being unable to continue the employment in which he is engaged. One patient was a sentry on post at the time he was attacked, and so sudden and severe was the onset that he had to call for relief. However, many cases have a gradual onset, and it was not uncommon for men to report sick with a history of having felt ill for a day or two, or even three. The onset is usually manifested by pain in the loins, often also in the legs, with headache and fever. Frequently the sensation is one of extreme weariness, rather than of pain. Chilliness is at times, but not usually, complained of. The appetite is nearly always impaired, and vomiting or diarrhœa are occasional features.

Catarrhal symptoms, such as coryza or bronchitis, are not present, unless as a complication, and are usually due to preexisting causes. Sore throat is described as common in some epidemics. We observed it in very few cases, and consider it rare. The skin is usually much injected, especially over the head and neck. Injection of the conjunctiva and lachrymation are common signs; photophobia is uncommon. We have not seen jaundice of either skin or mucous membranes. The early injection of the skin is described by some authors as the primary eruption. We agree with Guiteras and Cartaya in thinking that this term should not be applied to it. There is, in practically all cases, but one eruption, and it appears later, if at all. We have seen one case in which two eruptions appeared, but it was the rare exception which only served to emphasize the rule.

In a few cases the onset is so gradual and its manifestations so mild that it may not be noticed at all. Case 8 of our experimental series, who also had an æstivo-autumnal malarial infection, is a case in point. The incubation period in this case and the date of the eruption indicate that he had had dengue for about four days, while a blood examination showed that his chill and high fever of November 25 were of malarial origin.

Fever.—Fever is in practically all cases present from the beginning, and in the majority it reaches its maximum within twenty-four hours. This primary rise may exceptionally be to 40.5° C. (105° F.), or even 41° C. (106° F.), usually it reaches to about 39.7° C. (103.5° F.). In a minority of cases the ascent is gradual (see Case 2).

By the end of twenty-four hours the temperature has usually fallen 1° C. (2° F.) or more, and the period of intermission has begun. In some cases this drop in temperature is delayed until the beginning of the third day, quite exceptionally the same high point may be reached on four or five successive days (see Case 9).

However, in the typical case the temperature has fallen as stated at the end of twenty-four hours. The fall may carry it to normal, or only as low as 37.8° C. (100° F.), 38.3° C. (101° F.), or 38.9° C. (102° F.). There it remains, usually until the fifth day, when it again rises to almost as high a point as its early maximum. On the sixth day there is generally a sudden fall, by crisis, to normal, and the disease is ended. Critical discharges do not, in our observation, usually attend this fall in temperature, though profuse perspiration may occur.

When the chart is "typical" it is very characteristic of the disease, and enables one to pronounce a correct diagnosis at sight. Often it is not typical. The sharp rise on the first day and another on the fifth or sixth day, occur sufficiently often, however, to make the temperature chart at least as characteristic as in many other diseases in which much diagnostic significance is attached to it, as in typhoid fever.

Guiteras and Cartaya (7) protest against the description of the disease as one characterized by two febrile paroxysms, and contend that the fever is one attack, usually lasting six days, and only exceptionally subsides to normal before the sixth day. We agree with them in this, though we see no more objection to speaking of two paroxysms in this disease, when the temperature does go to normal between them, than in speaking of paroxysms in malaria under similar conditions.

The variations of this "typical" temperature record are manifold, as is shown in very many charts in our possession. However, in the majority of instances the type may be recognized even through the variations.

Hyperpyrexia, causing dangerous symptoms, is mentioned as a rare occurrence. We have not observed it.

Meningeal symptoms may, according to Guiteras and Cartaya, so alter the chart as to make it unrecognizable. We have not seen such cases. Our most severe case, and the one in which we observed the most marked nervous symptoms, showed an almost "typical" chart (see Case 10).

Pulse.—The resemblance between beginning dengue and beginning yellow fever, and the dissociation of pulse and fever in the latter disease, give to the pulse of dengue an importance it would not otherwise merit. Guiteras and Cartaya, who studied and wrote of the disease with its differential diagnosis from yellow fever as their main theme, summarize their observations on the pulse by saying that "in general it is not slow as in yellow fever, and especially not in the first days, but that dengue shows a tendency to slow pulse."

We have seen in no case a markedly slow pulse, and think that in general the pulse follows the temperature fairly well, although the tendency to slowness is most apt to be manifested by a relatively small rise in pulse rate. Writing with little experience with yellow fever, we should consider the pulse a valuable diagnostic feature.

Pain.—Pain is usually described as the earliest symptom. This is true in nearly all cases, so far as the patient knows, but as before stated, it is often preceded for several hours by a rising temperature. The pain is frequently severe, infrequently excruciating and immediately disabling. Also in a few instances it is trifling and very rarely it may be absent. It is in nearly every case manifested as headache and almost as frequently as lumbar pain. In a smaller number, but still a large majority of all cases, it is also present in the limbs, especially in the calves of the legs; less often, but still not rarely there is abdominal pain.

The headache may be frontal, vertical, temporal, occipital or post-orbital. Of these varieties we should place frontal headache as first in order of frequency, post-orbital second, temporal third, and vertical and occipital as least frequent. Movement of the eyeballs is often a cause of pain, particularly in patients complaining of post-orbital pain.

The pains in the lumbar region, trunk and limbs are of varying severity, in many cases giving rise to most bitter complaint, in others only being mentioned in response to inquiry. Such inquiry will in the vast majority of instances, practically all, elicit an account of pain. This is described by Guiteras and Cartaya as being localized in the deep insertions of the muscles. This seems to be the condition at times, but almost as frequently the bodies of the muscles are affected, especially of those in the legs, where the fleshy calf is often very painful. In spite of the fact that the disease is called "break-bone fever," we have seldom had patients complain of pains in the bones.

Joint pains are not infrequently complained of, especially in the knees. In only one case did we see marked redness or swelling of the joints; in this one the wrists were involved.

Intercostal pain is very unusual and pain in the abdominal muscles is even less so; Guiteras and Cartaya likened the latter to the sensation produced by pressing a large iron on the abdomen. This description we have elicited a few times.

Skin eruption.—As previously stated, the face is usually deeply flushed and the eyes injected and watery at the onset of the disease. This appearance we have found very characteristic, and, in the circumstances under which we have worked, an almost pathognomonic sign. A similar appearance may be produced by so many beginning diseases that we would not give it any such weight where there was danger of confusing it with such diseases.

The redness may extend over the entire surface, but it is usually more marked on exposed parts, such as the face, neck, and hands. It is not a true eruption, but a general capillary dilatation and in appearance it resembles a mild sunburn, or the dilatation caused by a hot bath, rather than a scarlatinal rash. It may last for any length of time, from five or six hours to two or three days. It is not constantly observed and we have seen a few cases in which pallor was present instead.

We have in no case seen jaundice, neither did Guiteras and Cartaya, who, of course, kept it constantly in mind. These writers state that the skin is generally hyperæsthetic. We have not noticed such a condition and have had no complaints of it, so that we assume that it may vary in different epidemics.

The true rash undoubtedly varies greatly in the frequency of its occurrence, as well as in its duration and localization. We agree with Guiteras and Cartaya in regarding it as possibly present in all cases, though not noticed in all because it frequently is faint in appearance and of ephemeral duration. While we have not kept careful notes of all the patients we have examined, we think that we have seen the rash in about 75 per cent of our cases.

It most commonly appears about the fourth day, not infrequently with the terminal rise. At times we have seen it upon the third day, and at least twice in our experimental cases upon the second. As we received a majority of our cases, excepting those produced by inoculation, on the second, third, or fourth day, and as quite a number had the eruption when we first saw them, we could not determine accurately just when it did appear in some instances. We feel well satisfied, however, that the fourth or fifth day usually marks its first appearance.

The localization of the eruption varies. It occurs with greatest frequency, in our experience, on the trunk, either the anterior or posterior surface, or both, being involved. With this it may also appear on the wrists, ankles, neck, thighs, palms, or generalized over the entire body, the occurrence in the different locations being about in the order named.

The appearance of the rash also varies. The most common eruption more nearly resembles that of measles than any other well-known eruption, but it is not so dark in color, neither are the macules usually so coarse nor aggregated into such large patches. Another type resembles scarlatina, consisting of close set or coalescent, bright, red points, while between these two are intermediate types. Very rarely is the rash so vivid and plain as in scarlatina, measles or rubella. The measles-like eruption may be, at times, appreciable to the touch.

In some of the scarlatiniform eruptions the injection may be so intense as to produce capillary rupture and minute extravasations, which show on the bright-red background as small, purple dots. An eruption of this character is longer in fading than the others. These small extravasations are more commonly seen on the back and buttocks than elsewhere, possibly because of the greater heat and pressure to which these parts are subjected. In one patient (Case 10) these small extravasations apparently suppurated; at all events, an abundant crop of miliary pustules, 1 to 3 millimeters in diameter, appeared over the buttocks, where the extravasations were abundant. The pustules were not painful and gave rise to no symptoms.

Occasionally, the eruption leaves small areas of skin, from 1 to 2 centimeters in diameter, uninvolved, which then present somewhat the appearance of wheals on a blushing surface. We have not seen an urticarial eruption.

The duration of the rash usually varies with its intensity, the well-marked eruptions lasting longer than the others, and, as stated, the scarlatiniform rash with extravasations the longest. In one such case (Case 2) the eruption lasted eight days. We have seen no other in which it lasted so long, though we have observed others in which it was visible for a week. In most cases it lasts about two days; that is, it appears on the fourth day, or the fifth and disappears by the time the temperature falls, on the sixth day. In many cases it lasts only one day, or possibly less, being well marked one morning and absent the next. In about one-fourth of our cases it was never seen at all, and possibly did not occur.

The disappearance of the rash in a minority of cases is followed by a fine desquamation which will not be noticed unless watched for closely.

In a very small minority the desquamation is easily observed as fine, bran-like, but abundant, scales. In one patient whom we saw but did not have under our care, the skin of the hands, arms and feet came off in large strips, many of them an inch square.

Alimentary system.—The tongue in nearly all cases presents a characteristic appearance. At first it is covered by a light, creamlike coat which rapidly thickens and darkens in the middle, disappearing from the edges; during the rest of the attack the tongue usually presents a heavy, yellowish

central coat, with a red tip and edges. It remains moist throughout and shows no tendency to fissure. The breath is heavy and at times foul, especially in cases showing constipation.

The appetite is practically always impaired or absent for the first few days. By the third or fourth day most of our patients were very hungry and asked for full diet, which all but two or three of them relished.

Nausea and vomiting occurred in a few cases, as did diarrhœa. This last was profuse and watery, but we never saw either mucus or blood in the stools, though both are said to occur at times. The vomiting and diarrhœa which we observed always occurred at the onset of the disease, and not as manifestations of the crisis.

As a rule, slight constipation is present, necessitating the administration of laxatives. As our patients were all soldiers leading active lives and taking much exercise, it is not improbable that the inaction of hospital confinement had as much influence as the disease in producing the constipation.

Nervous symptoms.—The most constant of these, the pains, have already been discussed. In three cases we have had delirium, that in one was very mild, in another slightly more marked, and in the third attended with marked hysterical symptoms and hallucinations. In all three cases the delirium was observed only at night, and in two it occurred as the patient was falling to sleep and may have been merely the manifestation of troubled dreams. The other case, Case 10, was as severe a one as we saw, but even in it the symptoms pointed rather to hysteria than to meningitis, and we afterwards learned that the patient had for several years, been subject to nervous attacks, beginning when he was a small boy and continuing until about two years ago. He also had an attack, similar to the one he showed during his fever, a short time after his return to duty. In this attack he got out of bed, ran about the room, shouted, wept and talked to his mother, who was of course not present. He was quieted, and the next day was impressed with the folly of his conduct and the necessity for maintaining self-control. Since that time, now nearly three months, he has had no trouble, and has performed his full duty.

We have not seen the "meningeal type" of the disease as it has been described by others, nor have we heard of any such cases occurring during this epidemic. We have not seen peripheral neuritis, unless some of the pains were due to such lesions, in which case the neuritis did not outlast the other symptoms and must have been trifling.

Insomnia was frequently observed while the fever was high and the pain severe. It was so evidently dependent on these causes and disappeared so soon that it did not require treatment.

Disturbances of the circulatory apparatus should probably be considered here. A considerable minority of patients complained of pain or dis-

comfort in the præcordium or of a sense of suffocation, and one man had an attack of syncope while eating. In none of these cases were we able to discover any sign of heart, lung, or pericardial lesion. Not even the cardiac rhythm was disturbed during any of our examinations.

However, the frequency of complaint, and the fact that one man actually fainted, show that the circulation is often disturbed in this disease, and this disturbance prompts us to join with other writers in pointing out the necessity for guarding against accidents from such a cause.

Hæmorrhages.—Several writers speak of the tendency to hæmorrhages, and Guiteras and Cartaya, whose cases were many of them sent to the hospital as yellow fever suspects, noted it in almost a fifth of the cases they studied. We did not observe hæmorrhages in any instance, if we except the small capillary ruptures described as an occasional feature of the eruption.

The possibility of the occurrence of hæmorrhages from other parts should be kept in mind, especially in yellow fever countries, for though Guiteras and Cartaya observed none from the stomach or bowels, other writers say they may occur.

Lymphatic glands.—It is stated by some observers that the lymphatic glands show an enlargement during this disease. This observation we can not confirm, as we saw no lymphatic enlargements except in cases which had shown them prior to the onset of dengue, or who developed inguinal adenitis from coexisting venereal disease.

Blood.—The results of the examination of the blood have already been considered.

Urine.—Guiteras and Cartaya state that albumen may often be found in the urine if very delicate tests are used, they detecting it in 41 per cent of their cases. We have never seen any symptoms referable to the urinary system except in a few men who had a coexisting gonorrhœa, and we therefore did not have the urine examined in many instances. We had eight urines examined, and they were all normal but one. However, our experience indicates that in some epidemics the condition of the urine gives more valuable information for a differential diagnosis between dengue and yellow fever than Guiteras and Cartaya thought.

Convalescence.—Many writers state that convalescence is often prolonged and tedious, and apt to be protracted by such complications as boils, joint affections, muscular pains, or weakness in the knees. In all of our cases convalescence has been prompt, practically all patients expressing a desire to return to duty as soon as the temperature fell.

Mortality.—All observers agree in saying that the mortality in dengue is so low as to be almost nothing. During the Australian epidemic 94 deaths occurred from the disease in Brisbane. The Robertson committee estimated that this represented about one death in 1,000 cases;

the mortality was relatively greater in females than in males, and was highest at the extremes of life; patients under 5 years of age contributing 37.6 per cent of all deaths, those over 60 years 35.5 per cent.

We have seen no deaths, and have heard of none during the epidemic we have studied. The disease does its harm from a military standpoint by disabling such large numbers of men at one time. When 600 men out of 1,000 or 1,200 are disabled for a period of at least a week each, the work of the command must, of course, suffer.

V. DIAGNOSIS.

As indicated in the consideration of the symptoms, the diagnosis of dengue will often not be made except in the presence of an epidmic, in which case the tendency would probably be to call any painful affection of sudden onset "dengue."

The fairly characteristic temperature chart, the sudden onset, severe pain, flushed face, the coated tongue, the eruption, and the leucopenia and lymphocytosis, unite to make the ordinary case easy of diagnosis, especially in the presence of an epidemic.

Care is required, under various conditions, in differentiating dengue from yellow fever, malaria, influenza, scarlet fever, measles, syphilis, tonsilitis, rheumatism, smallpox, and meningitis.

Yellow fever.—The differential diagnosis between yellow fever and dengue is probably the most important we have to consider, as the two diseases occur side by side in America, and mistaken diagnosis might lead to the gravest consequences, as a supposed dengue case is not apt to be so carefully guarded from mosquitoes as is a known one of yellow fever.

Guiteras and Cartaya, experienced in both diseases, say that the most valuable differential signs are the slower pulse, the jaundice and the haematemesis in yellow fever. None of these are apt to occur in dengue. Add to this the greater liability to albuminuria in yellow fever, the character of the prevailing epidemic, the mortality, the absence of the eruption and probably the blood examination, which in yellow fever does not show the characteristic leucopenia and lymphocytosis of dengue, and in the great majority of cases the diagnosis will be clear. Nevertheless, it would be the part of wisdom in all doubtful cases to act as though the disease were yellow fever.

Malaria.—The history and the microscope will usually make an early differentiation possible. In case they do not do so, quinine will do little harm.

Influenza.—The geographical and seasonal distribution of the two diseases do not correspond. Dengue occurs only with the mosquitoes, influenza where mosquitoes are absent and oftenest in cold weather. Influenza is usually accompanied by catarrhal symptoms, dengue rarely so, and then only accidentally. Dengue usually shows an eruption of a

scarlatinal or rubeoloid type, influenza does not. Leucopenia and lymphocytosis point more strongly to dengue.

Scarlatina.—The occurrence or nature of the epidemic, the seasonal occurrence, the almost entire absence of sore throat and cervical glandular swelling, the age of the patient, the less marked toxic symptoms, the temperature chart, the leucocyte count and the usually slight desquamation, will in nearly all instances set dengue apart from scarlatina.

Measles.—The more sudden onset of dengue, the greater pain, the absence of coryza, the appearance of the temperature chart, the epidemic and its season, usually makes this differentiation easy.

Syphilis.—Confusion with syphilis will occur but rarely, and only in individual cases. In such cases the history, the chart, the usually less violent onset of symptoms, the examination for chancre, mucous patches, etc., will practically always enable one to make a diagnosis.

Tonsilitis.—The onset of acute, follicular tonsilitis is at times, in its suddenness, its painfulness and fever, much like that of dengue. The examination of the throat is usually sufficient for the making of a correct diagnosis.

Rheumatic fever.—Acute articular rheumatism is at times, but unusually, simulated by dengue. In the latter disease the joint involvement, when present, is less marked and more ephemeral, while the other dengue symptoms, especially the eruption, make the diagnosis clear.

Smallpox.—The sudden onset, the flushed face, the violent pain in the head and back, the high temperature, make the early stages of smallpox resemble those of dengue, and it is probable that smallpox developing during dengue epidemics will often be mistaken for it. The history of exposure to smallpox, the absence or great age of vaccination scars, would point to that disease, while the evolution of the pocks would soon put the case beyond the realm of doubt.

Meningitis.—Some of the cases of the "meningeal" type of dengue, as described by other writers, can probably only be differentiated from epidemic cerebro-spinal meningitis by the presence of the dengue epidemic and the result of the bacteriological examinations of the fluid obtained by spinal puncture.

VI. TREATMENT.

Prophylaxis.—We believe that our observations and the total failure of all our attempts to transmit the disease by fomites or contact indicate the character of the prophylactic measures. Protection from mosquitoes is probably all that is necessary, but there is the posibility that other transmission of infected blood may rarely occur, as for example through other insects, infected hypodermic needles, and in a few other possible but improbable ways. We believe that in the case of the military service the screening of barracks would prevent such epidemics as the one which occurred at Fort William McKinley, and it would appear that economy

would be subserved by the screening of all barracks and quarters in countries where malaria and dengue are prevalent.

Medicinal.—Our cases did not receive treatment except in a few instances and for special symptoms, as we wished to obtain a picture of the unaltered disease. To judge from our observations on these untreated cases we think that other than symptomatic treatment, to promote the patient's comfort, is not called for.

Cold bathing, sponging, and the use of ice-caps are advisable to keep the temperature within bounds; for the pain and nervous symptoms, opium and bromides would probably be safer routine measures than the use of the coal-tar products; because, first, in this disease they are more effective, and, second, they would be less apt to harm an already disturbed heart.

VII. CONCLUSION.

In concluding our report we desire to express our appreciation of the encouragement and aid rendered us by Maj. Gen. Leonard Wood, commanding the Philippines Division, without which it would have been impossible for us to have made these researches. We also desire to thank Dr. R. P. Strong, the Director of the Biological Laboratory of the Bureau of Science, for the use of apparatus, and Mr. Charles S. Banks, Entomologist of the Biological Laboratory, Bureau of Science, who rendered us assistance in the identification of mosquitoes.

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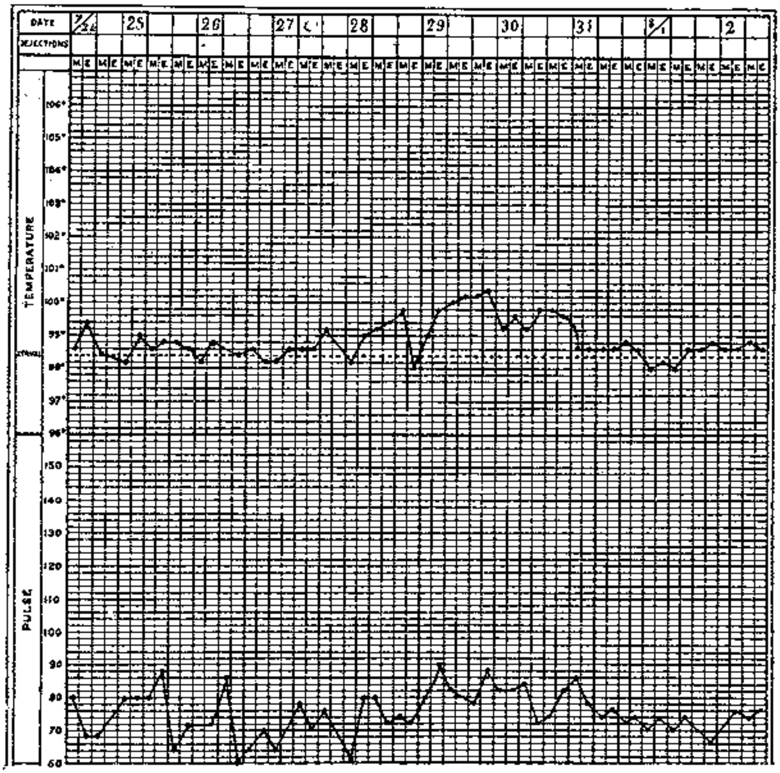
LIST OF CHARTS.



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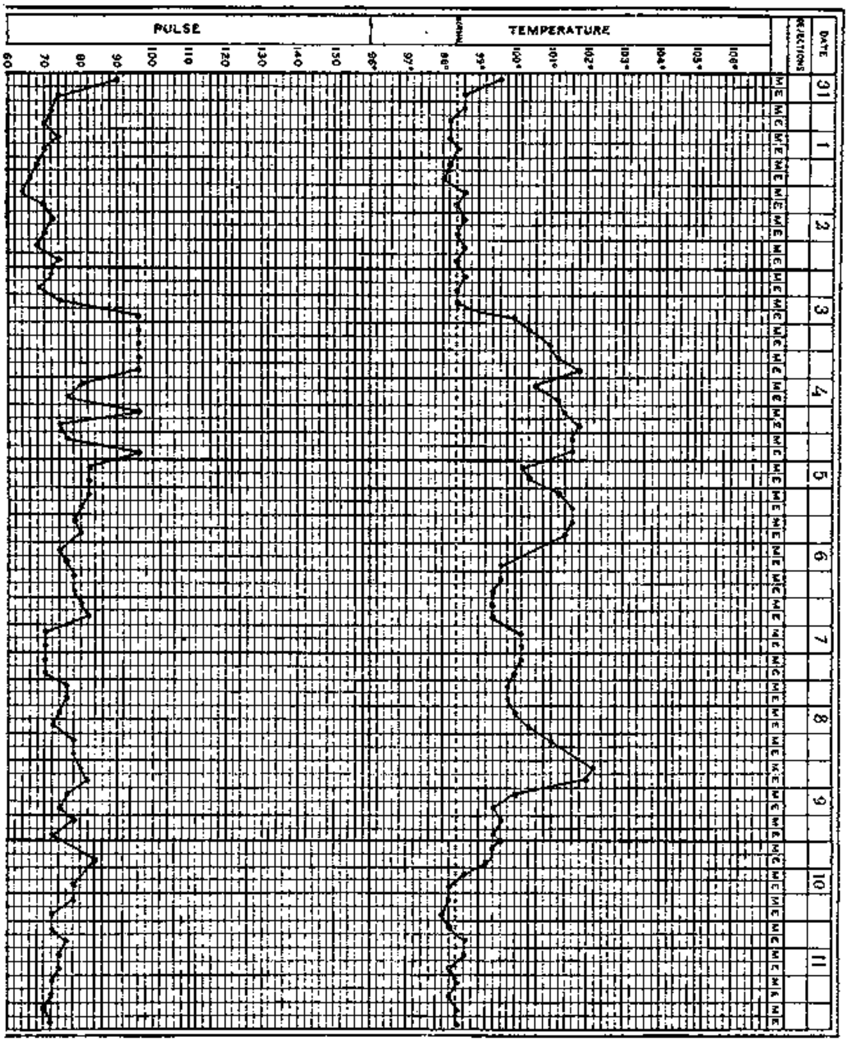

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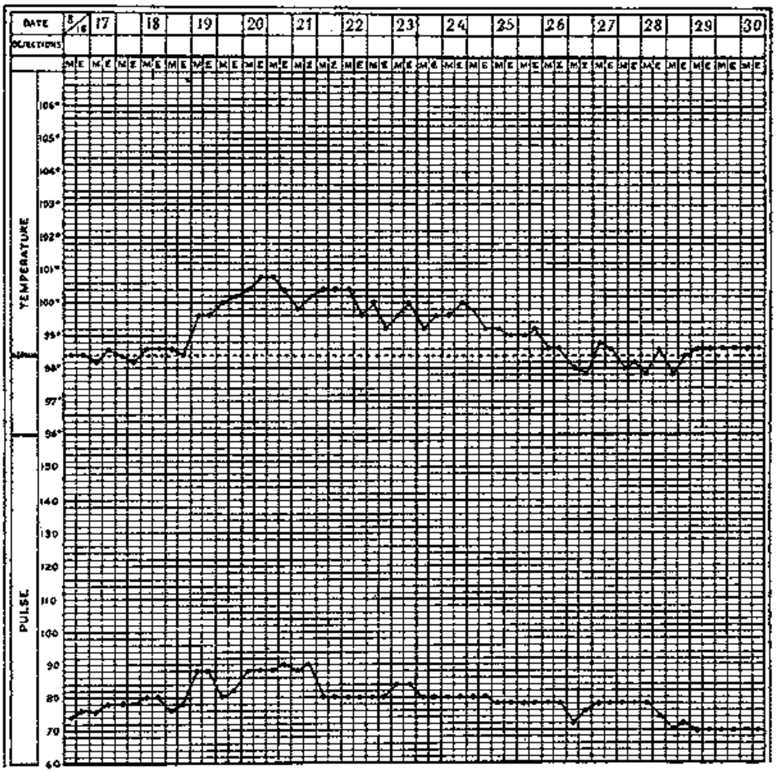

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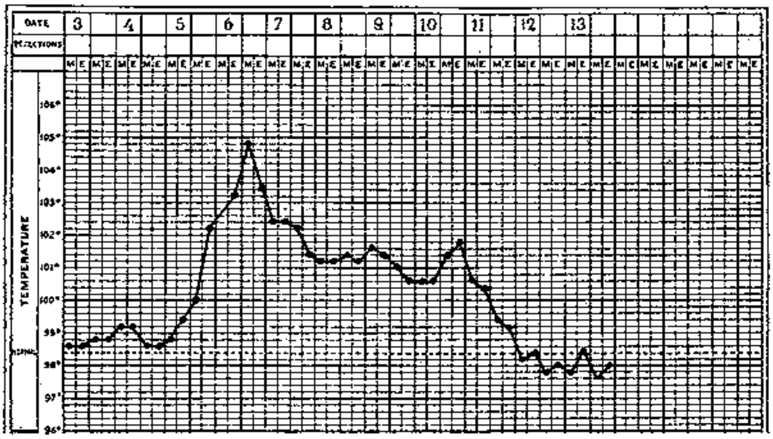

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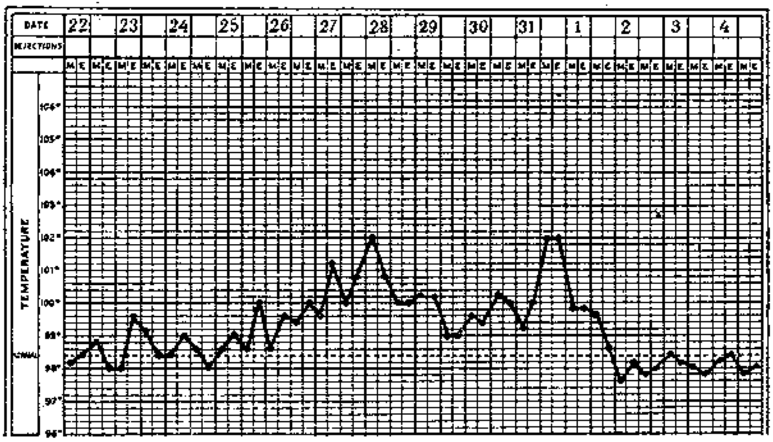

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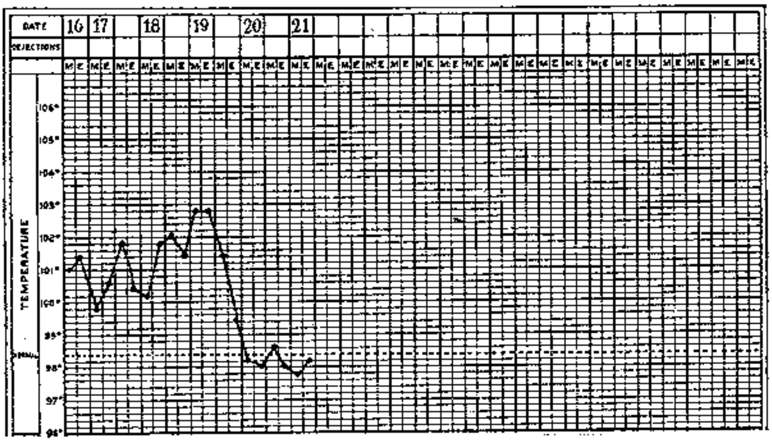

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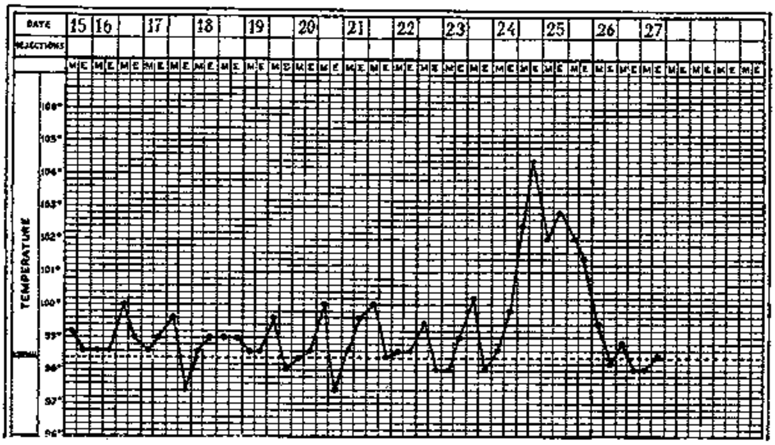

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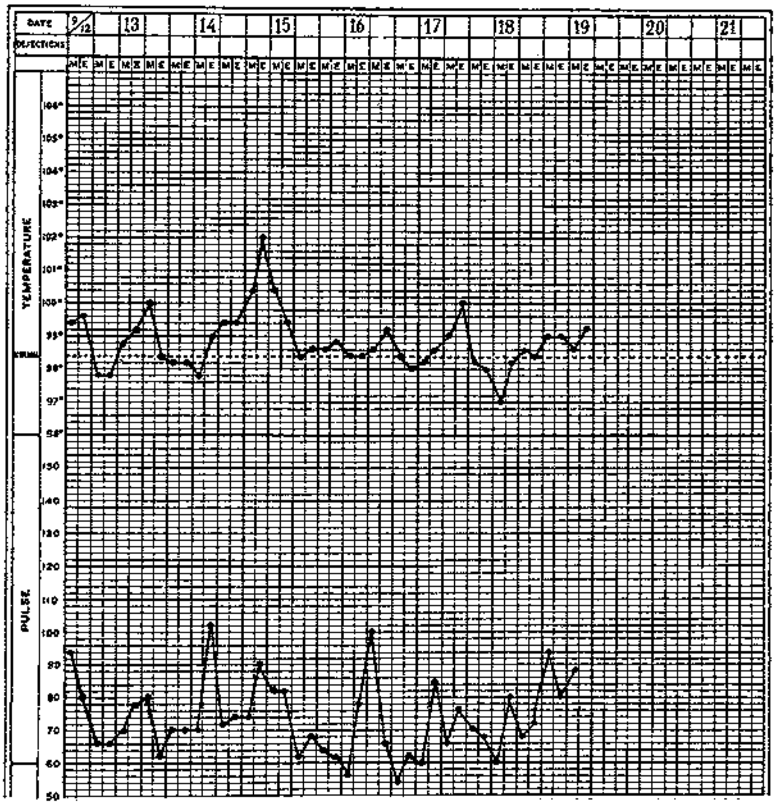

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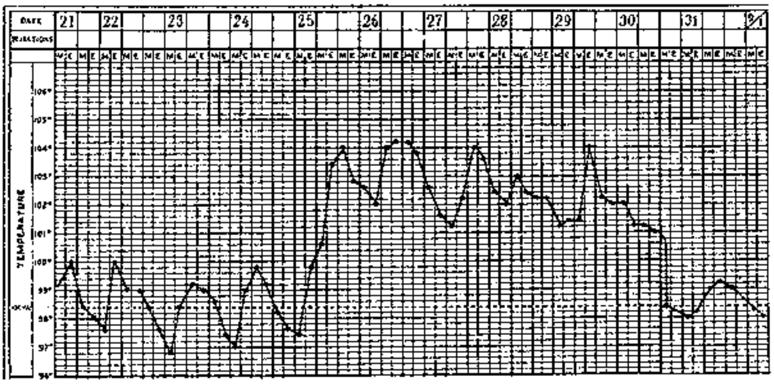

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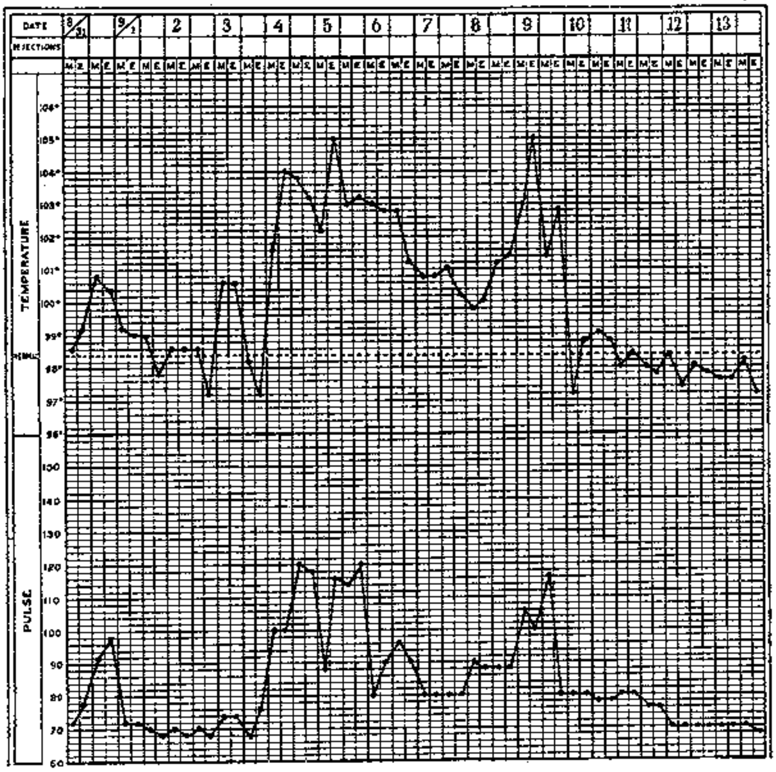
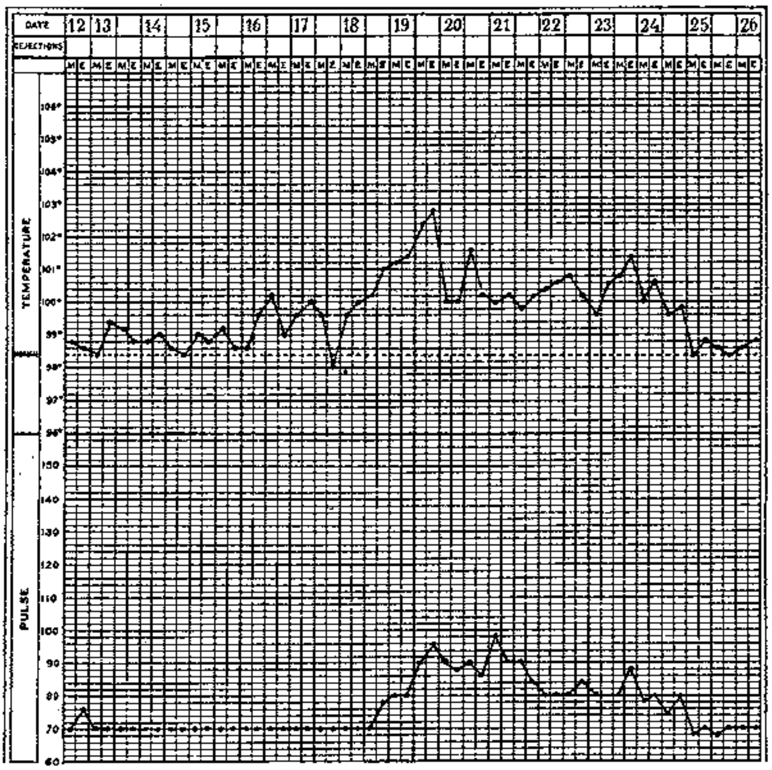


CASE 10, CHART 10.



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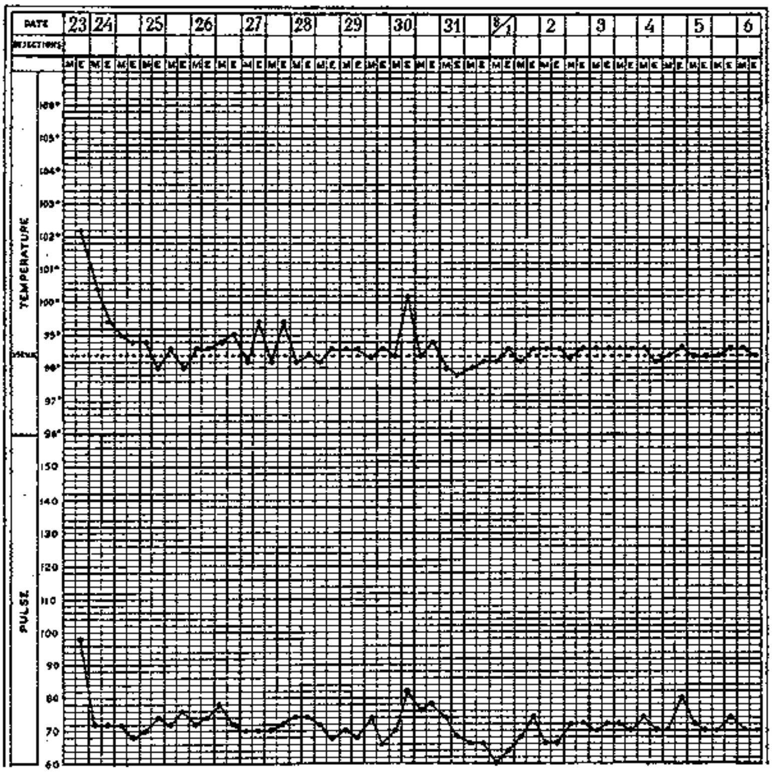

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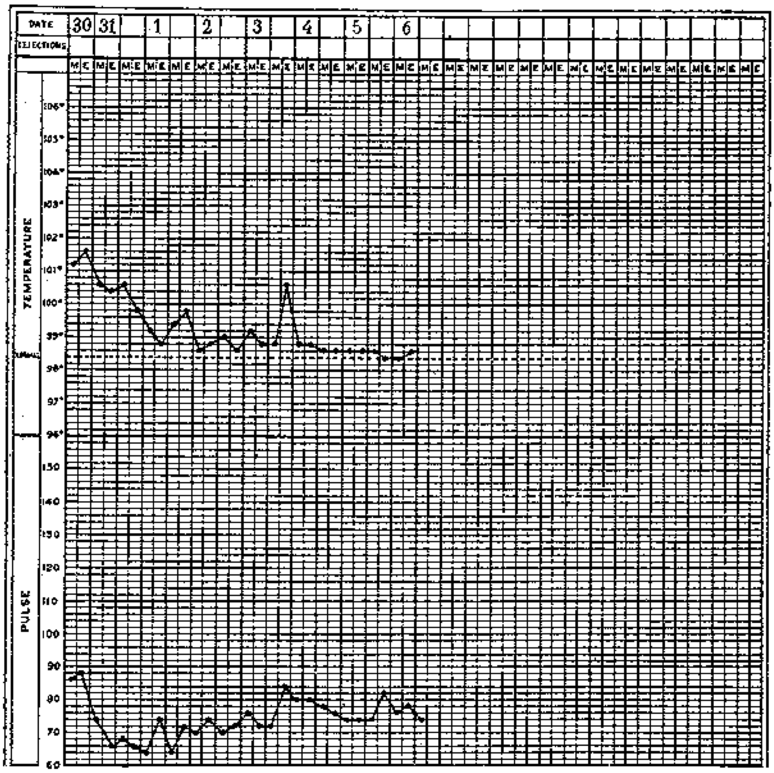

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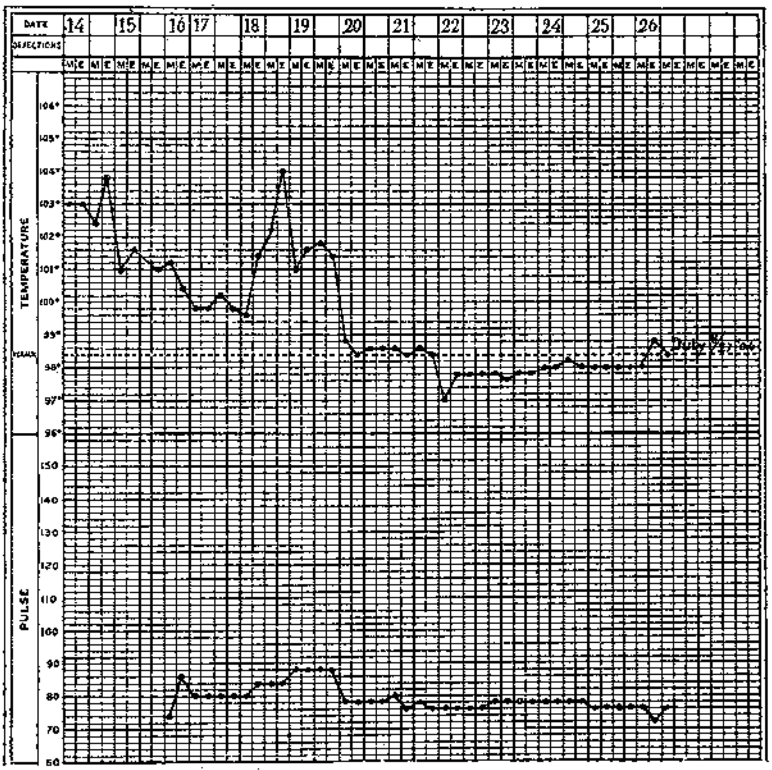

CASE 36, CHART C.

Case 13.—J. G., private, Company I, Sixteenth Infantry. This soldier belonged to a company of the Sixteenth Infantry that had furnished twelve cases to the hospital with dengue before this man volunteered. He had therefore been exposed to the disease.

Experiment 1: On September 12, 1906, the subject rinsed his mouth with normal salt solution containing 12 minims of dengue blood, our object being to determine if the dengue parasite could infect through an intact mucous membrance. The result of the experiment was negative.

Experiment 2: On September 19 the subject was given an intravenous inoculation of filtered blood from Case 11 (Chart 11). No symptoms developed.

Experiment 3: On the night of October 4 the subject was exposed to mosquitoes that had bitten Case 44 (Chart E) the night before, and was bitten at least twice. Dengue did not develop.

Experiment 4: On the night of October 15 he was bitten many times by mosquitoes that had bitten a dengue case two nights before. The result was negative.

Experiment 5: On October 22 the subject was given an intravenous inoculation of 20 minims of unfiltered blood from Case 95 (Chart S). No symptoms of dengue developed, and the man was returned to duty October 29, 1906.

Case 14.—J. B. P., private, Company M, Sixteenth Infantry. At the time of volunteering the company to which this man belonged had furnished ten men suffering from dengue to the Forth McKinley Hospital.

Experiment 1: On the night of September 24, 1906, the subject was exposed to mosquitoes that had bitten Case 11 (Chart 11) the night before. He was bitten several times and also many times during the next ten days. The result of the experiment was negative.

Experiment 2: The subject was exposed October 26 and 27 to mosquitoes that had bitten a typical case of dengue on October 25. The result of the experiment was negative.

Experiment 3: On November 17 the subject was given an intravenous inoculation of unfiltered blood from Case 82 (Chart N), who was suffering from a typical attack of dengue. No successful result was obtained in this experiment, and the man was returned to duty November 23, 1906.

Remarks.—These men were all exposed to fomites, in addition to the experiments outlined, and we believe that the results of these experiments demonstrate that absolute immunity to dengue is present in certain individuals.

9. CONTAGION IN DENGUE.

We have already noted the theories regarding the contagious character of dengue. We have carefully studied this portion of our subject, and believe that the following facts conclusively prove that dengue is not contagious in the least degree.

1. At the hospital at Fort William McKinley over 600 cases of dengue were treated in the general wards without a single case originating among the other patients in the wards. Only four men belonging to the Hospital Corps on duty at this hospital contracted the disease, three of them being nurses on night duty in the wards and the other a cook having no contact with the dengue patients. No precautions were used to prevent contagion other than the rigid use of mosquito nets at night, the dengue and other patients eating together, and being closely associated

throughout the day. It is noteworthy that the only men unprotected by the mosquito nets at night—that is, the three night nurses—all developed the disease.

2. In our dengue hospital, where we treated over 120 cases, no instance of infection occurred among the attendants, although their association with the dengue patients was very intimate and continued for over four months.

3. Our experiments with fomites were all negative. We endeavored to produce the disease by exposure of healthy men to fomites, the men experimented with living in mosquito-proof tents with patients suffering from dengue, throughout the entire course of the disease. They slept in their beds, wore their underclothing and pajamas, and ate and drank from the same table furniture. In this way we experimented with eight men, none of whom developed the disease from such exposure.

We conclude, therefore, that dengue is not a contagious disease, and that patients suffering from it may be placed in the general wards of a hospital without fear of infection, provided precautions are taken to protect the patients from mosquitoes.

Conclusions regarding the etiology of dengue.—From our study of the etiology of dengue, we believe the following conclusions are justified:

1. No organism, either bacterium or protozoön, can be demonstrated in either fresh or stained specimens of dengue blood with the microscope.

2. The red blood count in dengue is normal.

3. There occur no characteristic morphological changes in the red or white blood corpuscles in this disease.

4. Dengue is characterized by a well-marked leucopenia, the polymorphonuclear leucocytes being decreased, as a rule, while there is a marked increase in the small lymphocytes.

5. The intravenous inoculation of unfiltered dengue blood into healthy men is followed by a typical attack of the disease.

6. The intravenous inoculation of filtered dengue blood into healthy men is followed by a typical attack of the disease.

7. The cause of the disease is, therefore, probably ultramicroscopic.

8. Dengue can be transmitted by the mosquito, *Culex fatigans* Wied., and this is probably the most common method of transmission.

9. No organism of etiological significance occurred in bouillon or citrated blood cultures.

10. The period of incubation in experimental dengue averages three days and fourteen hours.

11. Certain individuals are absolutely immune to dengue, as proven by our experiments.

12. Dengue is not a contagious disease, but is infectious in the same manner as is yellow fever and malaria.

IV. SYMPTOMATOLOGY.

It is of cardinal importance in considering the symptoms and diagnosis of dengue to bear in mind the fact that it presents, in different epidemics and in different individuals in the same epidemic, a variety of clinical pictures; and that, while there is what may be called typical dengue, there are many variations from the type, and there is no one symptom that can be said to be pathognomonic, or even constant, if we except fever. We do not state positively that even fever is constant, but we are unable to satisfy ourselves that a given case is dengue unless it shows some fever, particularly at the onset. This doubtless accounts for the different descriptions of the disease that have been written. We agree with Guiteras and Cartaya in the belief that many cases can not be properly diagnosed except in the presence of an epidemic. We likewise agree with them that it is illogical to differentiate subtypes of the disease according to the dominant symptom, so we shall content ourselves with outlining the typical attack, and commenting on the usual symptoms. In doing this we will use the plan of the writers mentioned, whose observations and descriptions we consider accurate, clear and well balanced.

Invasion.—This is usually rather sudden, and, exceptionally in our experience, may be so sudden that the patient has to sit or lie down, being unable to continue the employment in which he is engaged. One patient was a sentry on post at the time he was attacked, and so sudden and severe was the onset that he had to call for relief. However, many cases have a gradual onset, and it was not uncommon for men to report sick with a history of having felt ill for a day or two, or even three. The onset is usually manifested by pain in the loins, often also in the legs, with headache and fever. Frequently the sensation is one of extreme weariness, rather than of pain. Chilliness is at times, but not usually, complained of. The appetite is nearly always impaired, and vomiting or diarrhœa are occasional features.

Catarrhal symptoms, such as coryza or bronchitis, are not present, unless as a complication, and are usually due to preëxisting causes. Sore throat is described as common in some epidemics. We observed it in very few cases, and consider it rare. The skin is usually much injected, especially over the head and neck. Injection of the conjunctiva and lachrymation are common signs; photophobia is uncommon. We have not seen jaundice of either skin or mucous membranes. The early injection of the skin is described by some authors as the primary eruption. We agree with Guiteras and Cartaya in thinking that this term should not be applied to it. There is, in practically all cases, but one eruption, and it appears later, if at all. We have seen one case in which two eruptions appeared, but it was the rare exception which only served to emphasize the rule.

In a few cases the onset is so gradual and its manifestations so mild that it may not be noticed at all. Case 8 of our experimental series, who also had an æstivo-autumnal malarial infection, is a case in point. The incubation period in this case and the date of the eruption indicate that he had had dengue for about four days, while a blood examination showed that his chill and high fever of November 25 were of malarial origin.

Fever.—Fever is in practically all cases present from the beginning, and in the majority it reaches its maximum within twenty-four hours. This primary rise may exceptionally be to 40.5° C. (105° F.), or even 41° C. (106° F.), usually it reaches to about 39.7° C. (103.5° F.). In a minority of cases the ascent is gradual (see Case 2).

By the end of twenty-four hours the temperature has usually fallen 1° C. (2° F.) or more, and the period of intermission has begun. In some cases this drop in temperature is delayed until the beginning of the third day, quite exceptionally the same high point may be reached on four or five successive days (see Case 9).

However, in the typical case the temperature has fallen as stated at the end of twenty-four hours. The fall may carry it to normal, or only as low as 37.8° C. (100° F.), 38.3° C. (101° F.), or 38.9° C. (102° F.). There it remains, usually until the fifth day, when it again rises to almost as high a point as its early maximum. On the sixth day there is generally a sudden fall, by crisis, to normal, and the disease is ended. Critical discharges do not, in our observation, usually attend this fall in temperature, though profuse perspiration may occur.

When the chart is "typical" it is very characteristic of the disease, and enables one to pronounce a correct diagnosis at sight. Often it is not typical. The sharp rise on the first day and another on the fifth or sixth day, occur sufficiently often, however, to make the temperature chart at least as characteristic as in many other diseases in which much diagnostic significance is attached to it, as in typhoid fever.

Guiteras and Cartaya (7) protest against the description of the disease as one characterized by two febrile paroxysms, and contend that the fever is one attack, usually lasting six days, and only exceptionally subsides to normal before the sixth day. We agree with them in this, though we see no more objection to speaking of two paroxysms in this disease, when the temperature does go to normal between them, than in speaking of paroxysms in malaria under similar conditions.

The variations of this "typical" temperature record are manifold, as is shown in very many charts in our possession. However, in the majority of instances the type may be recognized even through the variations.

Hyperpyrexia, causing dangerous symptoms, is mentioned as a rare occurrence. We have not observed it.

Meningeal symptoms may, according to Guiteras and Cartaya, so alter the chart as to make it unrecognizable. We have not seen such cases. Our most severe case, and the one in which we observed the most marked nervous symptoms, showed an almost "typical" chart (see Case 10).

Pulse.—The resemblance between beginning dengue and beginning yellow fever, and the dissociation of pulse and fever in the latter disease, give to the pulse of dengue an importance it would not otherwise merit. Guiteras and Cartaya, who studied and wrote of the disease with its differential diagnosis from yellow fever as their main theme, summarize their observations on the pulse by saying that "in general it is not slow as in yellow fever, and especially not in the first days, but that dengue shows a tendency to slow pulse."

We have seen in no case a markedly slow pulse, and think that in general the pulse follows the temperature fairly well, although the tendency to slowness is most apt to be manifested by a relatively small rise in pulse rate. Writing with little experience with yellow fever, we should consider the pulse a valuable diagnostic feature.

Pain.—Pain is usually described as the earliest symptom. This is true in nearly all cases, so far as the patient knows, but as before stated, it is often preceded for several hours by a rising temperature. The pain is frequently severe, infrequently excruciating and immediately disabling. Also in a few instances it is trifling and very rarely it may be absent. It is in nearly every case manifested as headache and almost as frequently as lumbar pain. In a smaller number, but still a large majority of all cases, it is also present in the limbs, especially in the calves of the legs; less often, but still not rarely there is abdominal pain.

The headache may be frontal, vertical, temporal, occipital or post-orbital. Of these varieties we should place frontal headache as first in order of frequency, post-orbital second, temporal third, and vertical and occipital as least frequent. Movement of the eyeballs is often a cause of pain, particularly in patients complaining of post-orbital pain.

The pains in the lumbar region, trunk and limbs are of varying severity, in many cases giving rise to most bitter complaint, in others only being mentioned in response to inquiry. Such inquiry will in the vast majority of instances, practically all, elicit an account of pain. This is described by Guiteras and Cartaya as being localized in the deep insertions of the muscles. This seems to be the condition at times, but almost as frequently the bodies of the muscles are affected, especially of those in the legs, where the fleshy calf is often very painful. In spite of the fact that the disease is called "break-bone fever," we have seldom had patients complain of pains in the bones.

Joint pains are not infrequently complained of, especially in the knees. In only one case did we see marked redness or swelling of the joints; in this one the wrists were involved.

Intercostal pain is very unusual and pain in the abdominal muscles is even less so; Guiteras and Cartaya likened the latter to the sensation produced by pressing a large iron on the abdomen. This description we have elicited a few times.

Skin eruption.—As previously stated, the face is usually deeply flushed and the eyes injected and watery at the onset of the disease. This appearance we have found very characteristic, and, in the circumstances under which we have worked, an almost pathognomonic sign. A similar appearance may be produced by so many beginning diseases that we would not give it any such weight where there was danger of confusing it with such diseases.

The redness may extend over the entire surface, but it is usually more marked on exposed parts, such as the face, neck, and hands. It is not a true eruption, but a general capillary dilatation and in appearance it resembles a mild sunburn, or the dilatation caused by a hot bath, rather than a scarlatinal rash. It may last for any length of time, from five or six hours to two or three days. It is not constantly observed and we have seen a few cases in which pallor was present instead.

We have in no case seen jaundice, neither did Guiteras and Cartaya, who, of course, kept it constantly in mind. These writers state that the skin is generally hyperæsthetic. We have not noticed such a condition and have had no complaints of it, so that we assume that it may vary in different epidemics.

The true rash undoubtedly varies greatly in the frequency of its occurrence, as well as in its duration and localization. We agree with Guiteras and Cartaya in regarding it as possibly present in all cases, though not noticed in all because it frequently is faint in appearance and of ephemeral duration. While we have not kept careful notes of all the patients we have examined, we think that we have seen the rash in about 75 per cent of our cases.

It most commonly appears about the fourth day, not infrequently with the terminal rise. At times we have seen it upon the third day, and at least twice in our experimental cases upon the second. As we received a majority of our cases, excepting those produced by inoculation, on the second, third, or fourth day, and as quite a number had the eruption when we first saw them, we could not determine accurately just when it did appear in some instances. We feel well satisfied, however, that the fourth or fifth day usually marks its first appearance.

The localization of the eruption varies. It occurs with greatest frequency, in our experience, on the trunk, either the anterior or posterior surface, or both, being involved. With this it may also appear on the wrists, ankles, neck, thighs, palms, or generalized over the entire body, the occurrence in the different locations being about in the order named.

The appearance of the rash also varies. The most common eruption more nearly resembles that of measles than any other well-known eruption, but it is not so dark in color, neither are the macules usually so coarse nor aggregated into such large patches. Another type resembles scarlatina, consisting of close set or coalescent, bright, red points, while between these two are intermediate types. Very rarely is the rash so vivid and plain as in scarlatina, measles or rubella. The measles-like eruption may be, at times, appreciable to the touch.

In some of the scarlatiniform eruptions the injection may be so intense as to produce capillary rupture and minute extravasations, which show on the bright-red background as small, purple dots. An eruption of this character is longer in fading than the others. These small extravasations are more commonly seen on the back and buttocks than elsewhere, possibly because of the greater heat and pressure to which these parts are subjected. In one patient (Case 10) these small extravasations apparently suppurated; at all events, an abundant crop of miliary pustules, 1 to 3 millimeters in diameter, appeared over the buttocks, where the extravasations were abundant. The pustules were not painful and gave rise to no symptoms.

Occasionally, the eruption leaves small areas of skin, from 1 to 2 centimeters in diameter, uninvolved, which then present somewhat the appearance of wheals on a blushing surface. We have not seen an urticarial eruption.

The duration of the rash usually varies with its intensity, the well-marked eruptions lasting longer than the others, and, as stated, the scarlatiniform rash with extravasations the longest. In one such case (Case 2) the eruption lasted eight days. We have seen no other in which it lasted so long, though we have observed others in which it was visible for a week. In most cases it lasts about two days; that is, it appears on the fourth day, or the fifth and disappears by the time the temperature falls, on the sixth day. In many cases it lasts only one day, or possibly less, being well marked one morning and absent the next. In about one-fourth of our cases it was never seen at all, and possibly did not occur.

The disappearance of the rash in a minority of cases is followed by a fine desquamation which will not be noticed unless watched for closely.

In a very small minority the desquamation is easily observed as fine, bran-like, but abundant, scales. In one patient whom we saw but did not have under our care, the skin of the hands, arms and feet came off in large strips, many of them an inch square.

Alimentary system.—The tongue in nearly all cases presents a characteristic appearance. At first it is covered by a light, creamlike coat which rapidly thickens and darkens in the middle, disappearing from the edges; during the rest of the attack the tongue usually presents a heavy, yellowish

central coat, with a red tip and edges. It remains moist throughout and shows no tendency to fissure. The breath is heavy and at times foul, especially in cases showing constipation.

The appetite is practically always impaired or absent for the first few days. By the third or fourth day most of our patients were very hungry and asked for full diet, which all but two or three of them relished.

Nausea and vomiting occurred in a few cases, as did diarrhœa. This last was profuse and watery, but we never saw either mucus or blood in the stools, though both are said to occur at times. The vomiting and diarrhœa which we observed always occurred at the onset of the disease, and not as manifestations of the crisis.

As a rule, slight constipation is present, necessitating the administration of laxatives. As our patients were all soldiers leading active lives and taking much exercise, it is not improbable that the inaction of hospital confinement had as much influence as the disease in producing the constipation.

Nervous symptoms.—The most constant of these, the pains, have already been discussed. In three cases we have had delirium, that in one was very mild, in another slightly more marked, and in the third attended with marked hysterical symptoms and hallucinations. In all three cases the delirium was observed only at night, and in two it occurred as the patient was falling to sleep and may have been merely the manifestation of troubled dreams. The other case, Case 10, was as severe a one as we saw, but even in it the symptoms pointed rather to hysteria than to meningitis, and we afterwards learned that the patient had for several years, been subject to nervous attacks, beginning when he was a small boy and continuing until about two years ago. He also had an attack, similar to the one he showed during his fever, a short time after his return to duty. In this attack he got out of bed, ran about the room, shouted, wept and talked to his mother, who was of course not present. He was quieted, and the next day was impressed with the folly of his conduct and the necessity for maintaining self-control. Since that time, now nearly three months, he has had no trouble, and has performed his full duty.

We have not seen the "meningeal type" of the disease as it has been described by others, nor have we heard of any such cases occurring during this epidemic. We have not seen peripheral neuritis, unless some of the pains were due to such lesions, in which case the neuritis did not outlast the other symptoms and must have been trifling.

Insomnia was frequently observed while the fever was high and the pain severe. It was so evidently dependent on these causes and disappeared so soon that it did not require treatment.

Disturbances of the circulatory apparatus should probably be considered here. A considerable minority of patients complained of pain or dis-

comfort in the præcordium or of a sense of suffocation, and one man had an attack of syncope while eating. In none of these cases were we able to discover any sign of heart, lung, or pericardial lesion. Not even the cardiac rhythm was disturbed during any of our examinations.

However, the frequency of complaint, and the fact that one man actually fainted, show that the circulation is often disturbed in this disease, and this disturbance prompts us to join with other writers in pointing out the necessity for guarding against accidents from such a cause.

Hæmorrhages.—Several writers speak of the tendency to hæmorrhages, and Guiteras and Cartaya, whose cases were many of them sent to the hospital as yellow fever suspects, noted it in almost a fifth of the cases they studied. We did not observe hæmorrhages in any instance, if we except the small capillary ruptures described as an occasional feature of the eruption.

The possibility of the occurrence of hæmorrhages from other parts should be kept in mind, especially in yellow fever countries, for though Guiteras and Cartaya observed none from the stomach or bowels, other writers say they may occur.

Lymphatic glands.—It is stated by some observers that the lymphatic glands show an enlargement during this disease. This observation we can not confirm, as we saw no lymphatic enlargements except in cases which had shown them prior to the onset of dengue, or who developed inguinal adenitis from coexisting venereal disease.

Blood.—The results of the examination of the blood have already been considered.

Urine.—Guiteras and Cartaya state that albumen may often be found in the urine if very delicate tests are used, they detecting it in 41 per cent of their cases. We have never seen any symptoms referable to the urinary system except in a few men who had a coexisting gonorrhœa, and we therefore did not have the urine examined in many instances. We had eight urines examined, and they were all normal but one. However, our experience indicates that in some epidemics the condition of the urine gives more valuable information for a differential diagnosis between dengue and yellow fever than Guiteras and Cartaya thought.

Convalescence.—Many writers state that convalescence is often prolonged and tedious, and apt to be protracted by such complications as boils, joint affections, muscular pains, or weakness in the knees. In all of our cases convalescence has been prompt, practically all patients expressing a desire to return to duty as soon as the temperature fell.

Mortality.—All observers agree in saying that the mortality in dengue is so low as to be almost nothing. During the Australian epidemic 94 deaths occurred from the disease in Brisbane. The Robertson committee estimated that this represented about one death in 1,000 cases;

the mortality was relatively greater in females than in males, and was highest at the extremes of life; patients under 5 years of age contributing 37.6 per cent of all deaths, those over 60 years 35.5 per cent.

We have seen no deaths, and have heard of none during the epidemic we have studied. The disease does its harm from a military standpoint by disabling such large numbers of men at one time. When 600 men out of 1,000 or 1,200 are disabled for a period of at least a week each, the work of the command must, of course, suffer.

V. DIAGNOSIS.

As indicated in the consideration of the symptoms, the diagnosis of dengue will often not be made except in the presence of an epidmic, in which case the tendency would probably be to call any painful affection of sudden onset "dengue."

The fairly characteristic temperature chart, the sudden onset, severe pain, flushed face, the coated tongue, the eruption, and the leucopenia and lymphocytosis, unite to make the ordinary case easy of diagnosis, especially in the presence of an epidemic.

Care is required, under various conditions, in differentiating dengue from yellow fever, malaria, influenza, scarlet fever, measles, syphilis, tonsilitis, rheumatism, smallpox, and meningitis.

Yellow fever.—The differential diagnosis between yellow fever and dengue is probably the most important we have to consider, as the two diseases occur side by side in America, and mistaken diagnosis might lead to the gravest consequences, as a supposed dengue case is not apt to be so carefully guarded from mosquitoes as is a known one of yellow fever.

Guiteras and Cartaya, experienced in both diseases, say that the most valuable differential signs are the slower pulse, the jaundice and the haematemesis in yellow fever. None of these are apt to occur in dengue. Add to this the greater liability to albuminuria in yellow fever, the character of the prevailing epidemic, the mortality, the absence of the eruption and probably the blood examination, which in yellow fever does not show the characteristic leucopenia and lymphocytosis of dengue, and in the great majority of cases the diagnosis will be clear. Nevertheless, it would be the part of wisdom in all doubtful cases to act as though the disease were yellow fever.

Malaria.—The history and the microscope will usually make an early differentiation possible. In case they do not do so, quinine will do little harm.

Influenza.—The geographical and seasonal distribution of the two diseases do not correspond. Dengue occurs only with the mosquitoes, influenza where mosquitoes are absent and oftenest in cold weather. Influenza is usually accompanied by catarrhal symptoms, dengue rarely so, and then only accidentally. Dengue usually shows an eruption of a

scarlatinal or rubeoloid type, influenza does not. Leucopenia and lymphocytosis point more strongly to dengue.

Scarlatina.—The occurrence or nature of the epidemic, the seasonal occurrence, the almost entire absence of sore throat and cervical glandular swelling, the age of the patient, the less marked toxic symptoms, the temperature chart, the leucocyte count and the usually slight desquamation, will in nearly all instances set dengue apart from scarlatina.

Measles.—The more sudden onset of dengue, the greater pain, the absence of coryza, the appearance of the temperature chart, the epidemic and its season, usually makes this differentiation easy.

Syphilis.—Confusion with syphilis will occur but rarely, and only in individual cases. In such cases the history, the chart, the usually less violent onset of symptoms, the examination for chancre, mucous patches, etc., will practically always enable one to make a diagnosis.

Tonsilitis.—The onset of acute, follicular tonsilitis is at times, in its suddenness, its painfulness and fever, much like that of dengue. The examination of the throat is usually sufficient for the making of a correct diagnosis.

Rheumatic fever.—Acute articular rheumatism is at times, but unusually, simulated by dengue. In the latter disease the joint involvement, when present, is less marked and more ephemeral, while the other dengue symptoms, especially the eruption, make the diagnosis clear.

Smallpox.—The sudden onset, the flushed face, the violent pain in the head and back, the high temperature, make the early stages of smallpox resemble those of dengue, and it is probable that smallpox developing during dengue epidemics will often be mistaken for it. The history of exposure to smallpox, the absence or great age of vaccination scars, would point to that disease, while the evolution of the pocks would soon put the case beyond the realm of doubt.

Meningitis.—Some of the cases of the "meningeal" type of dengue, as described by other writers, can probably only be differentiated from epidemic cerebro-spinal meningitis by the presence of the dengue epidemic and the result of the bacteriological examinations of the fluid obtained by spinal puncture.

VI. TREATMENT.

Prophylaxis.—We believe that our observations and the total failure of all our attempts to transmit the disease by fomites or contact indicate the character of the prophylactic measures. Protection from mosquitoes is probably all that is necessary, but there is the posibility that other transmission of infected blood may rarely occur, as for example through other insects, infected hypodermic needles, and in a few other possible but improbable ways. We believe that in the case of the military service the screening of barracks would prevent such epidemics as the one which occurred at Fort William McKinley, and it would appear that economy

would be subserved by the screening of all barracks and quarters in countries where malaria and dengue are prevalent.

Medicinal.—Our cases did not receive treatment except in a few instances and for special symptoms, as we wished to obtain a picture of the unaltered disease. To judge from our observations on these untreated cases we think that other than symptomatic treatment, to promote the patient's comfort, is not called for.

Cold bathing, sponging, and the use of ice-caps are advisable to keep the temperature within bounds; for the pain and nervous symptoms, opium and bromides would probably be safer routine measures than the use of the coal-tar products; because, first, in this disease they are more effective, and, second, they would be less apt to harm an already disturbed heart.

VII. CONCLUSION.

In concluding our report we desire to express our appreciation of the encouragement and aid rendered us by Maj. Gen. Leonard Wood, commanding the Philippines Division, without which it would have been impossible for us to have made these researches. We also desire to thank Dr. R. P. Strong, the Director of the Biological Laboratory of the Bureau of Science, for the use of apparatus, and Mr. Charles S. Banks, Entomologist of the Biological Laboratory, Bureau of Science, who rendered us assistance in the identification of mosquitoes.

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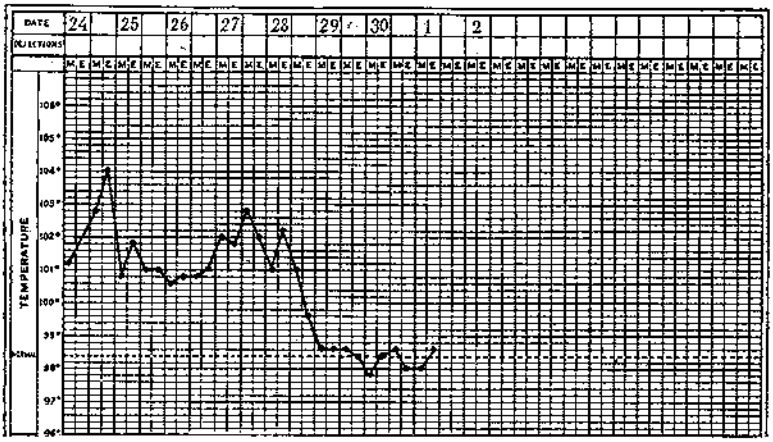
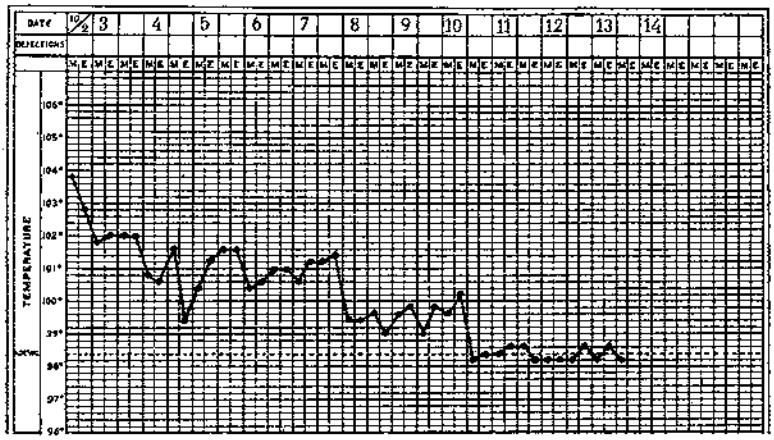


CASE 38, CHART D.



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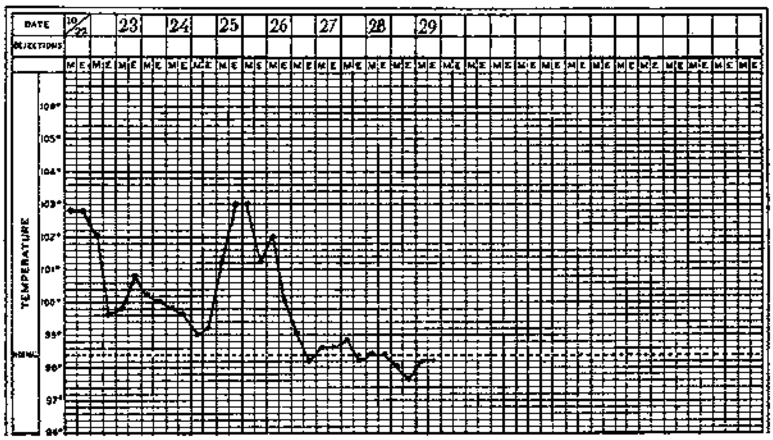

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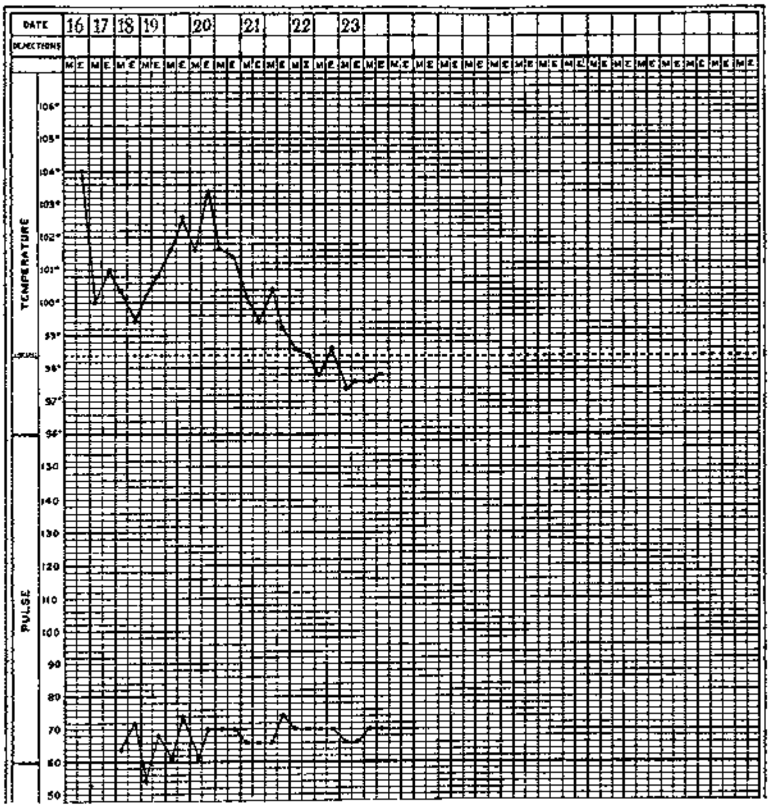

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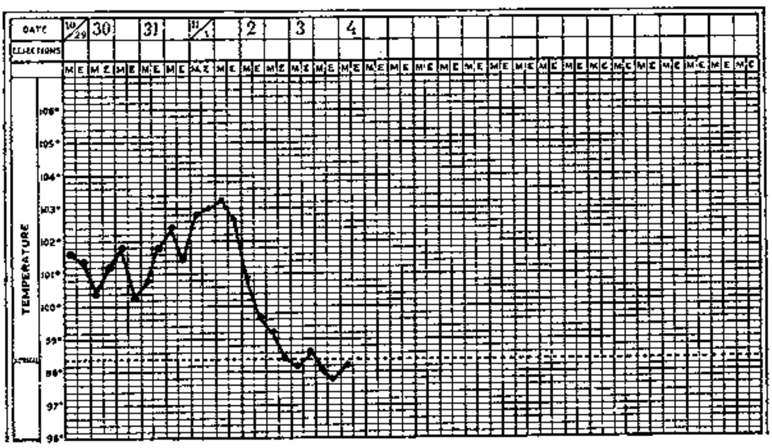

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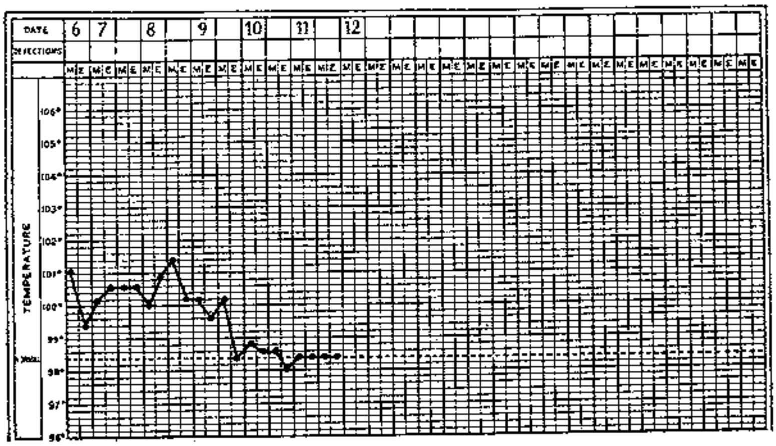

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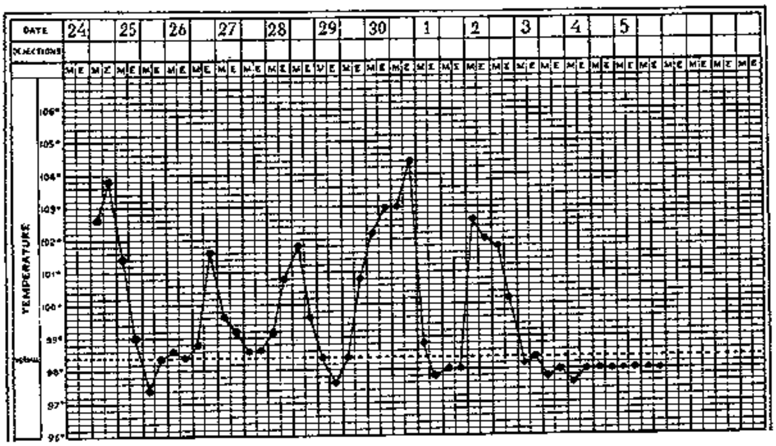

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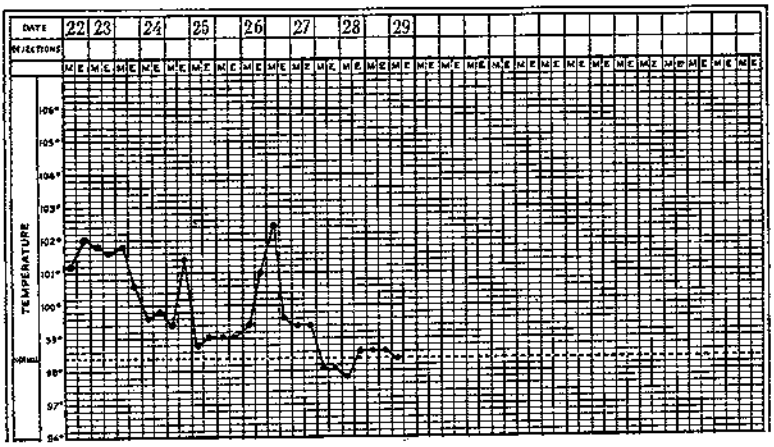

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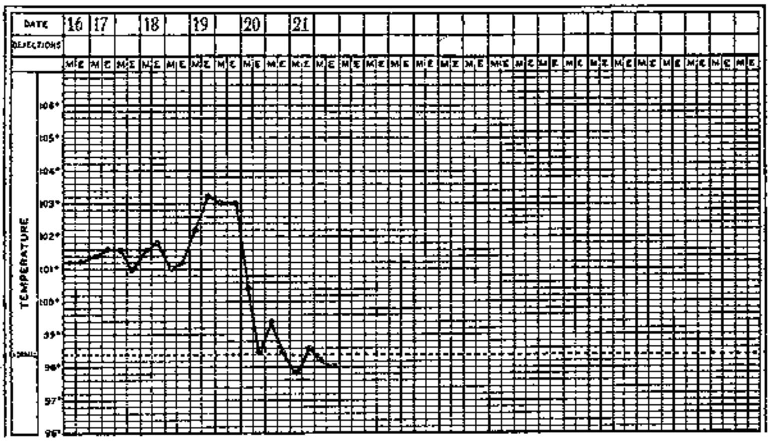

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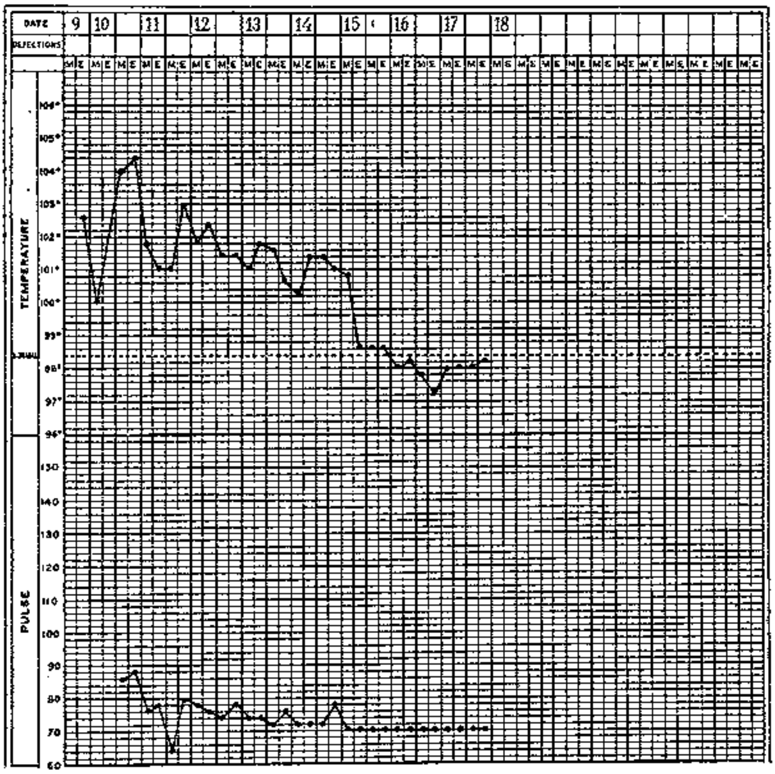

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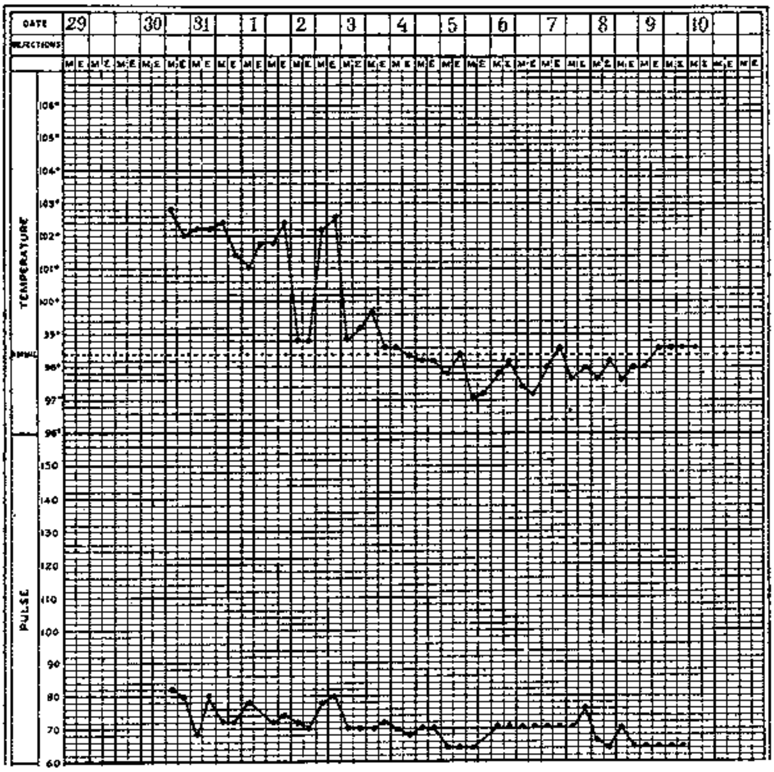

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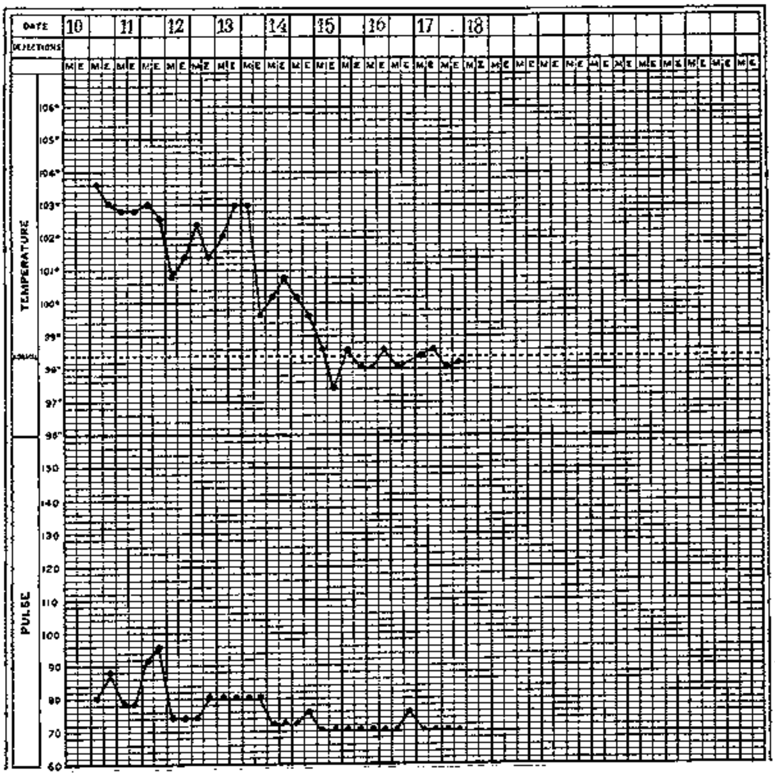

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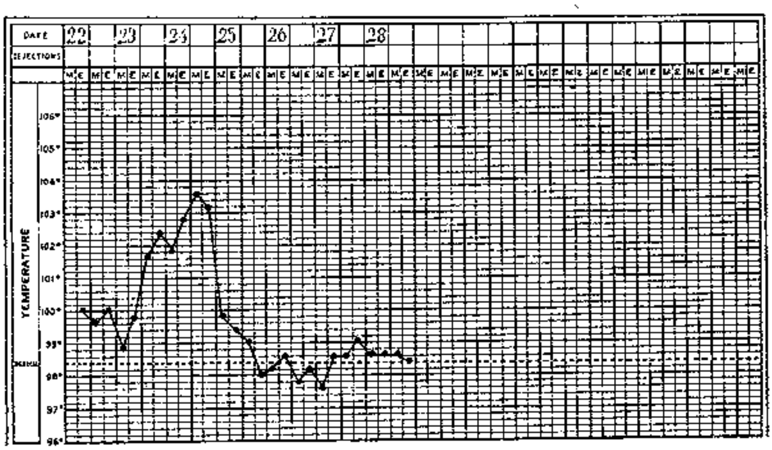


CASE 95, CHART S.

MAP.

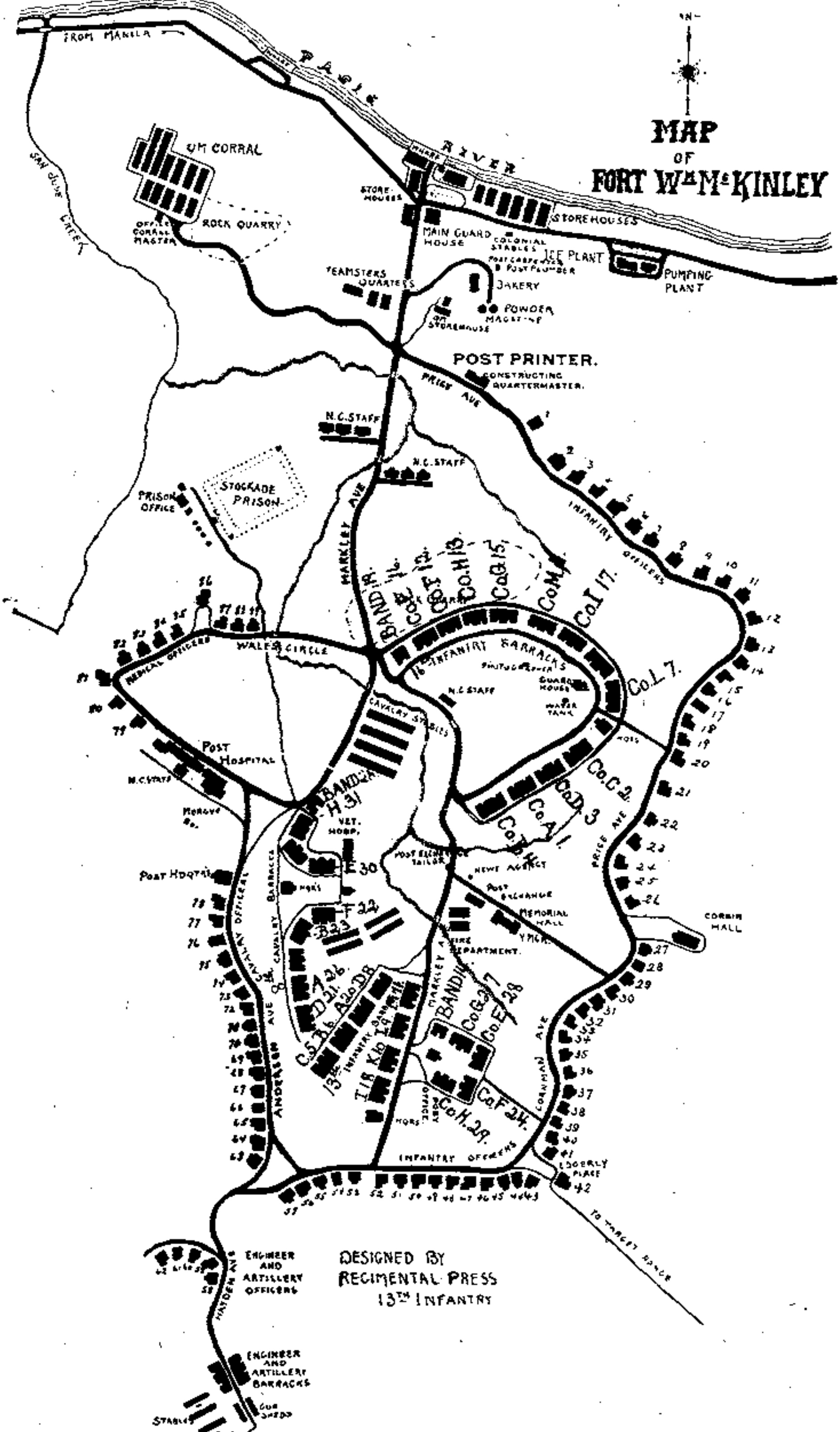
Sketch map of Fort William McKinley, Rizal Province, P. I. 151

EXPLANATION OF MAP.

The map is intended to illustrate the spread of the epidemic of dengue through the post of Fort William McKinley, Rizal Province, P. I.

The letters placed opposite the barracks indicate the company occupying them, and the figures accompanying the letters indicate the order in which the barracks were infected. It will be observed that barracks widely separated became infected before others in apposition, and that the last barracks to become infected were those of the Eighth Cavalry.

The red line indicates the course of a small stream of water which is an ideal breeding place for mosquitoes.


MAP
OF
FORT Wm. McKINLEY
N
FROM MANILA
PASIG RIVER
QM CORRAL
ROCK QUARRY
SAN JUAN CREEK
STORE HOUSES
STOREHOUSES
MAIN GUARD HOUSE
COLONIAL STABLES
ICE PLANT
PUMPING PLANT
TEAMSTERS QUARTERS
BAKERY
POWDER MAGAZINE
QM STOREHOUSE
POST PRINTER.
CONSTRUCTING QUARTERMASTER.
PRICE AVE
N.C.STAFF
N.C.STAFF
STOCKADE PRISON
PRISON OFFICE
MCKINLEY AVE
INFANTRY OFFICERS
BAND 16
Co.E 12
Co.F 12
Co.H 13
Co.G 15
Co.M
Co.I 17.
Co.L 7.
16 INFANTRY BARRACKS
GUARD HOUSE
WATER TANK
N.C.STAFF
WALES CIRCLE
MEDICAL OFFICERS
CAVALRY STABLES
POST HOSPITAL
N.C.STAFF
Co.C 2
Co.D 3
Co.A 1
Co.B 4
BAND 2
H 31
VET. HOSP.
E 30
F 22
B 23
A 26
D 21
A 20
D 8
C 5
B 6
POST HOSPITAL
CAVALRY OFFICERS
CAVALRY BARRACKS
ANDERSON AVE
NEWS AGENCY
POST EXCHANGE
MEMORIAL HALL
Y.M.C.A.
FIRE DEPARTMENT.
CORBIN HALL
BAND
Co.G 27
Co.E 28
INFANTRY BARRACKS
Co.F 24
Co.H 29
CORNMAN AVE
INFANTRY OFFICERS
LOCKERLY PLACE
TO TARGET RANGE
ENGINEER AND ARTILLERY OFFICERS
HAYDEN AVE
DESIGNED BY
REGIMENTAL PRESS
13TH INFANTRY
ENGINEER AND ARTILLERY BARRACKS
GUN SHEDS
STABLES

REVIEWS.

A Text-Book of Pathology. By Alfred Stengel, M. D. With 399 illustrations in the text, many of them in colors, and 7 full-page chromolithographic plates. Fifth edition, thoroughly revised. Cloth. Pp. 979. Price, $5.00 net; half morocco, $6.00 net. Philadelphia and London: W. B. Saunders Company, 1906.

It is obvious that a work upon pathology which has reached a fifth edition in a period of eight years must be possessed of more than ordinary merit, and have answered the needs of those who have consulted it. Written from the standpoint of the clinical pathologist, the subjects, in the work under review are presented in such a manner as to be of the greatest service to the practitioner and student of medicine, and taken as a whole, the work is one of the most valuable treatises upon pathology contributed by an American author. This edition is well up to date and much recent work has been incorporated or summarized; however, we greatly miss references to the literature of the various subjects discussed, especially in that portion of the work devoted to the animal and vegetable parasites, where a few well-chosen references would have proven of great value to the student.

The first six chapters of the work are devoted to general pathology, and are most excellent, especially the chapter upon progressive tissue changes, in which is given a beautifully illustrated and concise description of the histo-pathology of tumors.

Chapters 7 and 8 are devoted to the bacteria and the diseases due to them and to the animal parasites. In the reviewer's opinion the inclusion in a text-book of pathology of anything like an adequate consideration of the vegetable and animal parasites infecting man is impossible and might better be altogether omitted, but, unfortunately, it has become a custom to attempt to do so, and the chapters referred to are very satisfactory and the subjects are presented in an attractive manner; the author has accepted the classification of amœbæ as defined by Schaudinn, and includes the *Spirochætæ* under the bacteria. As regards the position of the *Spirochætæ*, it would have been better to have regarded their biological position as uncertain, especially as the recent work of Breinl appears to have disproven that of the adherents of the bacterial theory and again swung the pendulum toward the Protozoa. The illustrations in this section of the work are as a rule good, but many of them are reproductions of

old and imperfect woodcuts; the photomicrograph given of *Treponema pallidum* could much better have been used to represent "*refringens*" for it is not at all typical of "*pallidum.*"

The remainder of the work is devoted to special pathology, is well illustrated, and presents the subject in an interesting and instructive manner; owing, undoubtedly, to lack of space, many important subjects are merely mentioned, or the descriptions are so brief as to be of little practical value. The pathology of the diseases of the Tropics has suffered more in this way than any other branch of medicine, as an instance of which may be mentioned the description of the pathology of dysentery; no distinction is made between the pathology of specific or bacillary dysentery and the amœbic variety, and the Shiga bacillus is not even mentioned by name, but referred to as "a bacillus resembling the typhoid bacillus." It is unfortunate that so excellent a work as the one before us should be marred by so unsatisfactory a description of the pathology of this important group of infections.

The book is carefully indexed and is well printed and bound.

C. F. C.

The Elements of the Science of Nutrition. By Graham Lusk, Ph. D., M. A., F. R. S. (Edin.) Illustrated. Cloth. Pp. 326. Price $2.50 net. Philadelphia and London: W. B. Saunders Company, 1906.

Professor Lusk has given a very clear and comprehensive review of the known facts about the very complex subject of nutrition, including the results of his own careful work. The book deals largely with the scientific side of the subject and contains little of the practical details usually prominent in books upon this subject. It is to be hoped that the study of such books as this one together with the works of Pawlaw, Atwater, Chittenden and others who are doing so much toward elucidating the problems of nutrition will lead to the more general practice of this subject upon a scientific basis.

W. E. M.